

ES&H manual

Environment, Safety, and Health

Volume II

Part 13: Biological

Document 13.6

Safe Handling and Use of Biological Research Materials

Recommended for approval by the ES&H Working Group

Approved by: Glenn L. Mara
Deputy Director for Operations

New document or new requirements

Approval date: November 25, 2002
Editorial Update: December 3, 2003

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This work performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under Contract W-7405-ENG-48.

13.6

Safe Handling and Use of Biological Research Materials***Contents**

1.0	Introduction	1
1.1	Applicability	1
2.0	Hazards	2
2.1	Biohazards.....	3
2.1.1	Biohazardous Materials	3
2.1.2	Biohazardous Agents.....	4
2.2	Human Subjects.....	6
2.3	Human Blood and Bodily Fluids	6
2.4	Genomic Materials	6
2.5	Cell Culture Lines	7
2.6	Animals.....	7
2.7	Hazardous Materials	7
3.0	Controls	8
3.1	Administrative Controls	8
3.1.1	Institutional Review.....	8
3.1.2	Protocol Review	10
3.1.3	Work Practice Controls	10
3.1.4	Medical Waste Management.....	12
3.1.5	Accidents.....	13
3.1.6	Procurement.....	13
3.1.7	Transfer of Select Agents	13
3.1.8	Transportation	14
3.1.9	Medical Surveillance	14
3.1.10	Training	15
3.2	Engineered Controls.....	15
3.3	Personal Protective Equipment.....	17
4.0	Responsibilities.....	17
4.1	Workers	18
4.2	Facility Points of Contact	18
4.3	Responsible Individuals.....	18
4.4	Authorizing Individuals	18
4.5	Payroll Supervisors.....	19
4.6	LLNL Biosafety Operations Committee	19
4.7	LLNL Institutional Biosafety Committee	20

* Editorial Revision

4.8 Health Services Department.....	20
4.9 Hazards Control Department.....	21
4.10 Biosafety Officer	21
4.11 Environmental Protection Department	22
4.11.1 Environmental Analysts	22
4.11.2 Hazardous Waste Management Technicians.....	22
5.0 Work Standards.....	23
5.1 Work Smart Standards	23
5.2 Other Requirements.....	23
6.0 Resources for More Information.....	24
6.1 Contacts	24
6.2 Lessons Learned	24
6.3 Other Sources.....	24

Appendices

Appendix A Acronyms, Terms, and Definitions	25
Appendix B Biosafety Levels.....	31
Appendix C Classification of Human Etiologic Agents	37
Appendix D Select Agents.....	48
Appendix E Priority Categories Used for Select Agents by the Centers for Disease Control and Prevention	50
Appendix F USDA-Regulated Materials	52
Appendix G Universal Precautions.....	56
Appendix H Disinfectants	58
Appendix I Biological Safety Cabinets.....	67
Appendix J Vacuum Line Filters.....	71

Tables

Table B-1. Biological research laboratory containment levels of the Centers for Disease Control and National Institutes of Health	32
Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins. (cont'd.)	60
Table I-1. Performance guidelines for biological safety cabinets.	68

Figure

Figure 1. Biohazard symbol.	12
Figure J-1. Protective apparatus for vacuum system.	71

13.6

Safe Handling and Use of Biological Research Materials

1.0 Introduction

The environment, safety, and health (ES&H) policy of Lawrence Livermore National Laboratory (LLNL) requires operations to be planned and performed safely, with full consideration for the law and for the protection of employees, the public, and the environment. This document in addition to other applicable documents in the *ES&H Manual* constitutes the biosafety manual for LLNL and describes the safety requirements for the conduct of all biological research operations. The requirements address operational hazards, environmental concerns, controls, and the responsibilities of personnel.

One objective of this document and other applicable documents within the *ES&H Manual* is to define LLNL's biological safety program. (The term "biological safety" is used interchangeably with "biosafety.") A biosafety program is a complete program of administrative controls, engineered controls (e.g., containment), and personal protective equipment (PPE) for reducing the risk of disease to employees who face potential occupational exposure to infectious agents or biologically derived molecules. The biosafety program is intended to protect employees, coworkers, laboratory support workers, products, and the environment. (For a definition of the terms used in this document, refer to Appendix A.)

All biological research operations conducted at LLNL shall satisfy the requirements in this and other appropriate documents in the *ES&H Manual*. In accordance with Integrated Safety Management (ISM), such work is covered by an Integration Work Sheet (IWS) or safety plan that specifically assesses and references responsibilities, hazards, and the controls necessary to conduct the operation safely. For more information, see Document 2.2, "Managing ES&H for LLNL Work," in the *ES&H Manual*.

1.1 Applicability

This document governs biohazardous operations that are limited to biosafety level (BSL) 1 or 2 activities involving small-scale (i.e., nonproduction, research laboratory-scale) amounts of biological research materials. No work at BSL 3 or higher is currently allowed at LLNL. For more information about BSLs, refer to Appendix B.

The requirements of this document apply to:

- All research activities (including human-subjects research and environmental restoration) and facilities involving the handling, storage, onsite transportation, or use of biohazardous research materials (biohazardous research materials are defined in Section 2.0).
- All persons (including LLNL employees, subcontract workers, consultants, support and service staff, participating guests, short- and long-term visitors, and summer students) who enter or work in research areas where biological research materials are present.

The requirements of this document do not apply to the following nonresearch services provided by the organizations indicated:

- Health care (Health Services Department)
- Emergency response and cleanup (Fire Department, Protective Force Division)
- Environmental restoration (Environmental Protection Department)
- Sewer and water system maintenance (Plant Engineering)

2.0 Hazards

The hazards associated with working with biological research materials range from personal exposure to accidental environmental releases. Personal exposure may result from the handling of materials such as toxins, infectious agents, or animals. The degree of personal exposure depends on the infectious source or contaminated source, the individual's immune status, and efficiency of the transmission of infection. Personal exposure may have benign results or may cause a disease requiring medical treatment.

Accidental releases into the environment may cause an imbalance in the normal, established microflora in the affected area. Such imbalance may pose a health threat to the general public (particularly persons who are immunologically compromised) and may have an impact on agriculture and sewage treatment facilities, for instance. Because of the serious ramifications of employee exposure and accidental releases into the environment, control measures to prevent such events are required.

The categories of materials, discussed in this section, are as follows:

- Biohazards
 - Biohazardous materials

— Biohazardous agents

- Human subjects
- Human blood and bodily fluids
- Genomic materials
- Cell culture lines
- Animals
- Hazardous materials

2.1 Biohazards

A biohazard is any biological material or component that presents a risk of illness or injury to humans, plants, or animals. Biohazards can be divided into biohazardous materials and biohazardous agents, which are described in this section.

2.1.1 Biohazardous Materials

Biohazardous materials are not capable of self-replication and are the components of biological agents that present a risk of causing illness or injury to humans, plants, and animals. The types of biohazardous materials are as follows:

Biological Toxins. Biological toxins (also referred to as biotoxins) are nonliving toxic proteins that are naturally produced by many different types of living organisms. Biotoxins are:

- Thousands of times more toxic by mass than chemical warfare agents.
- Considered to pose the same level of risk as the microorganisms that produce them.
- Not themselves infectious or contagious after exposure. However, a biotoxin-producing organism may be infectious or contagious after exposure.

Endotoxins. Endotoxins, which occur in the outer membrane of certain gram-negative bacteria, are not secreted but are released only when the cells are disrupted or destroyed. Endotoxins are complex polysaccharide molecules that elicit an antigenic response, resulting in fever and altered resistance to bacterial infections. Exposure may cause toxic hemorrhagic shock and severe diarrhea.

Clinical and Diagnostic Specimens. A clinical or diagnostic specimen is any human or animal material (e.g., excreta, secretions, blood, components, tissue, and

tissue fluids) handled for the purposes of diagnosis, treatment, or research. Such specimens pose a unique hazard because of the material's unknown infectious nature.

2.1.2 Biohazardous Agents

Biohazardous agents are agents that are capable of self-replication and have the capacity to produce deleterious effects in other biological organisms (particularly humans), and are also referred to as etiologic agents. Such agents include (but are not limited to) viruses, prions, chlamydia, bacteria, fungi, yeast, algae, and plants.

Factors to be considered in determining the level of infectiousness of an agent include:

- Agent factors, e.g., virulence, pathogenicity, infectious dose, environmental stability, route of transmission, communicability, operations, quantity, and availability of vaccine or treatment.
- Agent products able to elicit toxicity, physiological activity, or allergenicity.

Classification of Etiologic Agents. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have classified biological agents into four risk groups (RGs) by the degree of hazard. A higher RG number indicates a higher level of hazard to a healthy human being.

- RG 1: Agents not associated with disease in healthy human adults.
- RG 2: Agents associated with human diseases that are rarely serious and for which preventive or therapeutic interventions are often available.
- RG 3: Agents associated with serious or lethal human disease for which preventive or therapeutic intervention may be available (i.e., high individual risk but low community risk).
- RG 4: Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic intervention are not available (i.e., high individual risk and high community risk).

Appendix C is a list of biological agents classified into the four RGs in accordance with the CDC and the NIH (see Section 6.3 for related references).

Note that RGs do not correspond to biosafety levels (BSLs). RGs are used to classify biological agents themselves, whereas a BSL indicates the level of containment that is required in an operation.

Select Agents. The term “select agent” refers to a microorganism (i.e., virus, bacterium, fungus, or rickettsia) or toxin that is listed in 42 CFR 73, “Possession, Use, and Transfer of Select Agents for Humans.” Select agents have been identified as potential weapons of mass destruction that can be used in bioterrorism activities. See

Appendix D for a detailed list. The term also includes recombinant organisms and molecules that fall under either of the following categories:

- Genetically modified microorganisms or genetic elements shown to produce or encode for a factor associated with disease.
- Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins listed in 42 CFR 73 or their subunits.

In addition, the CDC further classifies select agents into the following categories according to priority in public health preparedness efforts:

- Category A – Highest-priority agents
- Category B – Second highest-priority agents
- Category C – Third highest-priority agents

For a list of the agents in the above priority categories, see Appendix E.

Classification of Oncogenic Viruses. The National Cancer Institute (NCI) has defined oncogenic (i.e., potentially tumor-causing) viruses as a special class of etiologic agents that have been categorized into three risk levels: low, moderate, and high. For more information, refer to the *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (see Section 6.3).

Materials Regulated by the U.S. Department of Agriculture. Use, storage, disposal, and transport of animal and plant pathogens or toxins and soil potentially contaminated with animal or plant pathogens or toxins are regulated by the U.S. Department of Agriculture (USDA). The regulations are enforced by the USDA, as well as by the California Department of Food and Agriculture (CDFA) and county departments of agriculture. A permit is required for the transport, use, handling, or storage of these materials. For a list USDA-regulated materials, see Appendix F. For more information about USDA-regulated materials, contact the biosafety officer or see the following Internet address:

<http://www.aphis.usda.gov/ppq/permits/plantpest/pathogen.html>

For more information about the CDFA, see the following Internet address:

<http://www.cdca.ca.gov/>

2.2 Human Subjects

Work with human subjects involves the handling of potentially contaminated materials, e.g., blood, bodily fluids, and tissue. For more information regarding the potential hazards of working with such materials, see Section 2.3 of this document, as well as Document 13.2, “Exposure Control Plan: Working Safely with Blood and Bloodborne Pathogens,” and Document 13.4, “Research Involving Human Subjects,” in the *ES&H Manual* for more information on the topic.

2.3 Human Blood and Bodily Fluids

In accordance with Occupational Safety and Health Administration (OSHA) regulations, human blood and human bodily fluids shall be assumed to be contaminated with bloodborne pathogens. Bloodborne pathogens include, but are not limited to, the hepatitis B virus (HBV), hepatitis C virus, and human immunodeficiency virus (HIV). See Document 13.2 for more information.

2.4 Genomic Materials

Recombinant genomic materials include nucleic acids [e.g., deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)] and are defined as either of the following:

- Molecules that are constructed outside living cells by joining natural or synthetic DNA or RNA segments to DNA or RNA molecules that can replicate in a living cell.
- Molecules that result from the replication described above.

Synthetic or recombinant DNA or RNA molecules that are likely to yield a potential harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterparts and to be in the same RG as those of the organism from which the DNA originated. Work with genomic materials that are likely to yield a potential harmful polynucleotide or polypeptide requires additional controls or higher containment levels as determined by the ES&H Team industrial hygienist as part of the hazard analysis determination.

Recombinant DNA or RNA experiments may involve microbial, animal, or plant hosts and are defined as any experiments involving:

- Construction and handling of recombinant DNA or RNA molecules.

- Organisms and viruses containing recombinant DNA or RNA molecules.
- Gene therapy.

2.5 Cell Culture Lines

Cell culture lines are known to harbor latent viruses either inadvertently or because of deliberate experimental infections and therefore pose an undetected hazard. Established cell lines of subprimate or normal primate origin that do not harbor a primate virus and are not contaminated with bacteria, viruses, parasites, or fungi exceeding RG 1 should be handled in a BSL 1 containment facility. Primary and permanent human or animal cell lines should be assumed to carry RG 1 viruses unless known to be infected with a more hazardous agent.

Permanent human lymphocyte cell cultures or primate cell lines derived from lymphoid or tumor tissue should be handled as if known to harbor the Epstein-Barr virus, an RG 2 agent. In addition, the following cell lines shall be handled in a BSL 2 containment facility:

- All cell lines that have been converted, exposed, or transformed by a primate oncogenic virus.
- Cell lines with an American Type Culture Collection rating of BSL 2.
- All new cell lines of undetermined status.

Under no circumstances should anyone work with cells derived from themselves or from their first-degree relatives, because their immune system may not provide adequate protection against transplantation and growth of the cells.

2.6 Animals

Working with animals poses a unique hazard to the animals themselves and to personnel handling the animals. Special care should be taken to limit exposure to biological agents that are zoonotic (i.e., readily communicable from animals to humans). For more information about the hazards associated with working with vertebrate animals, see Document 13.5, "Vertebrate Animals Used in Research," in the *ES&H Manual*.

2.7 Hazardous Materials

Working with biological materials usually involves working with radioactive and hazardous materials. The hazards of dealing with radioactive and hazardous materials are addressed in other *ES&H Manual* documents, e.g., those in Part 14

(Chemical) and Part 20 (Ionizing Radiation/Nonionizing Radiation). The hazards associated with such materials shall be considered during the review and authorization of work with biological research materials.

3.0 Controls

This section describes the three types of controls for work involving biological materials: administrative controls (e.g., protocol and institutional review), engineered controls (e.g., containment), and PPE.

Containment is the safe management of infectious (e.g., biological) or hazardous (e.g., radioactive) materials in a laboratory where such materials are handled or maintained. The purpose of containment is to reduce or eliminate exposure of Laboratory employees, other persons, and the outside environment to potentially infectious or hazardous agents. Containment requirements vary depending on the hazard present. For information about biological containment requirements or biological containment levels, see Appendix B. The terms “biological containment levels” and “biosafety level” are used interchangeably.

3.1 Administrative Controls

All activities involving biohazards research activities shall be authorized through the IWS process in accordance with ISM. For more information about ISM, see Document 2.1, “Laboratory and ES&H Policies, General Worker Responsibilities, and Integrated Safety Management,” in the *ES&H Manual*.

In addition to an IWS, a safety plan is also required when working with:

- Regulated materials, such as
 - Select agents (including their genomic materials).
 - USDA-regulated materials (e.g., animals, plants, and soil).
 - Bloodborne pathogens.
 - Recombinant DNA or RNA.
- Large-scale quantities (i.e., quantities greater than 10 liters) of any biological research material
- Genomic materials coding for virulence or toxin factors

3.1.1 Institutional Review

Work involving biohazardous materials shall be initially reviewed by the LLNL Biosafety Operations Committee (LBOC) as part of the work authorization process.

Additional review may be required depending on the specific hazards involved. The institutional committees' guidelines and forms are discussed below.

LLNL Biosafety Operations Committee. The purpose of LBOC is to facilitate the institutional review of proposed work with biological research materials.

LBOC is comprised of representatives of organizations such as the following:

- The Health Services, Hazards Control, and Environmental Protection Departments
- The Nonproliferation, Arms Control, and International Security Directorate
- The Chemistry and Materials Science Directorate
- The Biology and Biotechnology Research Program Directorate
- Institutional Biosafety Committee (IBC)

See Section 4.6 for the responsibilities of LBOC.

Institutional Biosafety Committee. The purpose of the IBC is to review LLNL research protocols to ensure that:

- Safe recombinant DNA methods and restrictive use of recombinant materials for human therapy and environmental applications are in accordance with the *NIH Guidelines for Research Involving Recombinant DNA* (see Section 5.2).
- All biohazardous research protocols involving etiologic agents and toxins adhere to the safety requirements specified in the CDC's *Biosafety in Microbiological and Biomedical Laboratories* (see Section 5.1) and to all applicable regulations pertaining to work with etiologic agents and toxins.

The IBC also functions as a licensee for the Laboratory Registration/Select Agent Transfer Program. For more information, see the following Internet address:

<http://www.cdc.gov/od/ohs/lrsat.htm>

See Section 4.7 for the responsibilities of IBC.

Institutional Review Board (IRB). The purpose of the IRB is to review proposed work with human subjects or with tissue or data from human subjects. The IRB ensures that the rights of human subjects are protected and that human subjects give their informed, voluntary consent to participate in the research. For more information, see Documents 13.2 and 13.4.

Institutional Animal Care and Use Committee (IACUC). The purpose of IACUC is to review proposed experimental work with any vertebrate animal. IACUC coordinates the monitoring of the humane care of animals. For more information regarding IACUC approval, see Document 13.5. Appropriate animal BSLs are assigned according to the hazards involved. All work with live animals is restricted to the Animal Care Facility.

3.1.2 Protocol Review

The protocol review process is a process used by institutional committees, as well as programs to identify the following:

- Health hazards associated with the work
- Environmental concerns
- Medical surveillance requirements (see Section 3.1.9)
- Containment requirements
- Training needs
- Ethical concerns
- Regulatory requirements and applicable Work Smart Standards (WSSs) (see Section 5.0)
- Best management practices

The purpose of review is to determine whether employees are at risk, whether the potential benefits of the research outweigh the risk, and whether adequate provisions or controls have been made to minimize or mitigate the hazards involved. Research involving more than minimal risk may require more-frequent review and controls. Research involving the use of human blood or other human bodily fluids shall be handled in accordance with the guidelines and work practices outlined in Document 13.2.

3.1.3 Work Practice Controls

Work with biohazardous agents or materials onsite shall be limited to BSL 1 or 2 containment levels. Appropriate BSL work practices (outlined in Appendix B of this document) are to be followed. Work practices for biological materials include the following:

Universal Precautions. Universal precautions are an approach to infection control in which all human blood and certain human bodily fluids are treated as if known to be contaminated with bloodborne pathogens. Universal precautions should be

followed at all times when handling human blood and other bodily fluids. See Appendix G for details.

Handwashing. Frequent handwashing is encouraged. Hands shall be washed with soap after handling potentially infectious materials and before leaving the laboratory.

Decontamination. All surfaces shall be decontaminated before initiating any work and at the end of each workday. The three levels of biological decontamination of microorganisms are:

1. Sanitize – The general reduction of microorganisms by use of general cleaning agents.
2. Disinfect – The destruction of targeted organisms with the use of chemicals or physical agents. For example, table tops and surfaces can be decontaminated with disinfectants such as amphyll or broccal. (See Appendix H for more information about disinfectants.)
3. Sterilize – The complete destruction of all microbes. Sterilization is in general part of the total decontamination process.

Reusable, rigid, leakproof secondary containers used to hold biohazardous waste shall be thoroughly washed and decontaminated each time they are emptied, unless the surfaces of the container have been completely protected from contamination by disposable liners, bags, or other devices removed with the waste. Methods of decontamination are listed in Appendix H and in Document 13.1, “Biological Controls and Operations,” in the *ES&H Manual*. Emergency response procedures are discussed in Document 22.1, “Emergency Management,” in the *ES&H Manual*.

Biohazard Signs. Biohazard signs bearing the biohazard symbol (see Figure 1) shall be placed as a warning sign at the entry of each room where biohazardous materials of RG 2 or higher are handled or stored. The sign shall indicate the biohazard present, the persons responsible for the work, and any restrictions on access to the room. Biohazard signs should be placed at the entry of each room where RG 1 materials are handled or stored. See Document 13.1 for more information about signs and labels.



Figure 1. Biohazard symbol.

In addition, the standard Health Hazard Communication Notice door sign can also be used to indicate that a biohazard is present in a room or area. See Document 10.2, “LLNL Health Hazard Communication Program,” in the *ES&H Manual*.

3.1.4 Medical Waste Management

The LLNL main site is considered a large-quantity generator because 200 pounds or more of medical waste is normally generated within any month of a 12-month period. Medical waste is subject to regulations of the California Department of Health Services, which are enforced by the Alameda County Health Care Services Agency (in the case of the LLNL main site) and by the San Joaquin County Public Health Services (in the case of Site 300). For more information, see Document 36.1, “Waste Management Requirements,” in the *ES&H Manual*.

In general, the following types of medical waste shall be segregated into specific waste streams for proper disposal:

- Syringes and other sharps shall be segregated from solid or liquid biohazardous waste and placed in a properly labeled and approved sharps container. All sharps containers shall be disposed of when 3/4 full. Sharps containers are first autoclaved before final destruction by incineration. (See Section 3.2 for more information about sharps containers.)
- Biohazardous materials that are to be autoclaved shall be placed in properly labeled, leakproof containers containing labeled, autoclavable biohazard bags. Such waste shall be disposed of within 7 days if stored at room temperature or within 90 days if stored at 0°C. All such autoclaves

shall have a proper county-issued permit prior to use. Contact your area ES&H Team environmental analyst for more information.

- If biohazardous materials are to be chemically disinfected, contact your area ES&H Team industrial hygienist for more information regarding the disinfectant of choice, its contact time, and methods of disposal. Chemically treated artifacts should not be autoclaved because of the vapor hazards of chemical disinfectants. For more information regarding chemical disinfectants, see Appendix H of this document.

3.1.5 Accidents

In any laboratory operation, the possibility of a spill or other accident exists. A thorough understanding of the potential hazards of the experiment, as well as careful planning, helps minimize personal injury (e.g., needle sticks) and property damage from spills. Carefully following the controls specified in this document and applicable IWSs and safety plans reduces the probability of an accident and mitigates the consequences if an accident occurs.

Needle Sticks. Needle sticks shall be reported as a reportable injury to both the workplace supervisor and the Health Services Department. For more information about injury reporting, see Document 4.5, “Incidents—Notification, Analysis, and Reporting,” in the *ES&H Manual*.

Spills. In the event of a large-scale spill, call 911. For more information about spills, see Document 22.1.

3.1.6 Procurement

Procurement of any etiologic agent or material specified in Appendices C, E, or F shall be approved by the LLNL biosafety officer, who can be contacted through the local ES&H Teams. For more information, see Document 21.1, “Acquisition, Receipt, Transportation, and Tracking of Hazardous Material,” in the *ES&H Manual*.

3.1.7 Transfer of Select Agents

Because select agents have the potential for causing mass destruction or widespread disease in humans, the CDC has determined that the transfer of the agents from one geographic site to another poses a risk of transmission of disease and is therefore subject to 42 CFR 73.

The CDC requires that, prior to transferring a select agent, both the shipping and receiving parties complete the required sections of CDC Form EA-101, “Transfer of Select Agents.” The form, available through the IBC, lists the restricted agents and required information about the shipping and receiving parties, the requesting and

transferring facilities, the registration numbers of the transferring and receiving facilities, the name of restricted agent requested, and the proposed use of the agent. The form shall accompany the request or purchase order for obtaining these restricted agents. Copies shall be maintained by both the requesting and transferring facility and sent to a designated central repository at the CDC to be available to federal and authorized local law enforcement authorities.

Form EA-101 shall be completed before any select agent is shipped from LLNL to any other location for any reason. This requirement also applies to transfers to and from LLNL-related facilities, e.g., Site 300 or the Production Genome Facility.

3.1.8 Transportation

Biological material shall be transported in a manner that does not pose a threat of injury to personnel or damage to property. Transportation of such materials requires the use of both proper packaging and shipping.

Onsite transportation of biological materials is performed using labeled, leakproof secondary containers. The labeling requirements to be followed are the same as those for the transportation of biological waste. Hazardous materials, substances, and waste (including biological materials but excluding analytical samples) may not be transported in bicycle baskets, laboratory coats, automobiles, or personal vehicles. For more information, see Document 21.2, "Onsite Hazardous Material Packaging and Transportation Safety Manual," in the *ES&H Manual*.

Offsite transportation of articles or substance that are likely to pose a significant risk to health, safety, property, or the environment shall comply with the applicable regulations of the Department of Transportation, International Air Transport Association, USDA, and CDC. Offsite shipping of hazardous materials (i.e., chemical, biological, and radiological) shall comply with Document 21.1. For specific packaging and shipping procedures, contact either the LLNL Transportation Office or the Materials Management Department.

3.1.9 Medical Surveillance

The types of medical surveillance applicable to employees involved with biological materials may include:

- Preemployment and periodic screening
- Vaccination
- Serum banking
- Post-exposure medical surveillance

Staff who work with pathogens may be required to participate in the medical surveillance program. Staff who work with human blood or other human bodily fluids shall participate in the medical surveillance program. Details about participation in the medical surveillance program are determined in the work review process. For additional information, contact the Health Services Department, or see Document 10.1, "Occupational Medical Program," in the *ES&H Manual*.

3.1.10 Training

Employees whose primary job is to handle biological materials should be trained at the time of initial assignment. Individuals who work directly with bloodborne pathogens, human blood, human tissues, or other body fluids shall take course HS4400, "Working Safely with Blood and Bloodborne Pathogens," and its annual refresher, HS4400-RW. Work supervisors should provide additional training for specific job duties as appropriate. Employees who are new to the field of biological research or who provide direct ES&H support to programs that use biological materials are also recommended to take HS4430, "Biosurety for the Safety Professional" (or the directorate-specified equivalent).

3.2 Engineered Controls

Engineered controls are used to isolate or remove hazards from the workplace in order to reduce the potential for exposure. Engineered controls, in combination with safe work practices that alter the manner in which tasks are performed, are expected to be the primary means of eliminating or minimizing the risk of occupational exposure. Engineered controls for biological research materials include, but are not limited to, the following:

- **Mechanical aids**

Mouth pipetting is prohibited, and the use of mechanical aids to transfer potentially harmful (i.e., biohazardous) materials is strictly enforced. Personnel shall use such devices for all pipetting.

- **Dead air boxes**

Dead air boxes are commonly used to reduce the potential of contamination while diluting or transferring stock concentrations of biohazardous materials. Work areas shall be decontaminated before and after each use to reduce the likelihood of cross-contamination.

- **Fume hoods**

Fume hoods are commonly used in the laboratory to draw air away from the work area. Fume hoods shall not be used for long-term storage of materials and equipment. For more information, see Document 12.4,

“Work Enclosures and Local Exhaust Systems for Toxic and Radioactive Materials,” in the *ES&H Manual*.

- **Negative-air-flow units**

All animals injected with infectious materials or receiving mutagens or carcinogens added to their food or water are to be housed in negative-air-flow animal units. Cages are to be labeled as to the type of hazard present, amount and route of exposure, and the date of administration. Signs placed on each unit shall indicate whether the animals are currently being exposed, were exposed in the past, or have not been exposed.

- **Biological safety cabinets (BSCs)**

BSCs provide sterile laminar airflow onto the work surface, containment of aerosols or droplets, protection of materials, and (in some cases) protection of the user. Not all BSCs are designed primarily for protection of the user. There are three different classifications of BSCs: Classes 1 through 3. The performance of BSCs shall be verified at the time of installation, whenever they are moved, and annually thereafter. For more information regarding BSCs, see Appendix I of this document and Document 13.1.

- **Containment protection for vacuum systems**

The aspiration of either tissue culture media from monolayer cultures or supernatants from centrifuge samples into primary collection flasks is a common laboratory procedure. Protection shall be provided against drawing aerosols of hazardous chemical or biological materials or overflow fluid into the house vacuum system. Protection is provided by the use of an air filter in the line immediately leading into the house vacuum line and an overflow flask for liquids between the collection flask and the air filter. For an example of this assembly, see Appendix J.

- **Sharps containers**

Sharps containers are hard, puncture-proof containers designed to prevent contact with needles and other sharp objects placed inside. Sharps containers must be labeled with the words SHARPS CONTAINER and should be disposed of after becoming 3/4 full. See Document 36.1 for more information regarding waste disposal protocol.

- **Intrinsically safe needles**

Intrinsically safe hypodermic needles and syringes are designed to sheath the needle after use, help prevent needle sticks, and are to be used for all hypodermic needle applications, except as approved through the IWS process. All hypodermic needles and syringes must be disposed of in a container properly labeled with the words SHARPS CONTAINER.

3.3 Personal Protective Equipment

Identifying and understanding a workplace hazard and then matching the needed PPE to the hazard is the key to selecting effective and appropriate PPE. In most cases, the Hazard Assessment and Control (HAC) form is sufficient documentation for work involving the PPE covered in this document. Examples of PPE used when working with biological materials include the following:

- **Respiratory protection**

Respiratory protection is required in some cases. When a BSC is used, however, respiratory protection is considered unnecessary and is not recommended, unless specified by an ES&H Team industrial hygienist.

- **Laboratory coats**

Laboratory coats minimize skin exposure and protect street clothes from being contaminated. Laboratory coats shall be worn while working in a laboratory but are not to be worn outside of laboratory areas.

- **Gloves**

The use of gloves is required when splashing is anticipated. Glove selection is important when dealing with a variety of chemicals and biological materials.

- **Face protection**

Safety glasses, safety goggles, or a face shield, as appropriate, shall be worn while working with biological research materials in a laboratory. A face shield is required when splashing is anticipated.

For more information regarding the selection and use of PPE, see Document 11.1, "Personal Protective Equipment," in the *ES&H Manual*, or contact the area industrial hygienist.

4.0 Responsibilities

General responsibilities for all workers are described in Document 2.1. Specific responsibilities for key personnel are listed under each title in the following sections.

Each person is expected to make every reasonable effort to protect himself or herself and others from injury or illness. Facility occupants are expected to guide and govern visitors and to assist new or temporary occupants to understand and follow the applicable procedures. Personnel with any doubts regarding the safety of any phase of work shall check with the Responsible Individual.

For responsibilities of the IRB and IACUC, see Documents 13.1, 13.4, and 13.5.

4.1 Workers

Workers shall:

- Participate as appropriate in the LLNL medical surveillance program.
- Perform work safely.
- Report any needle sticks or occupational exposures immediately to the Health Services Department and to the ES&H Team.

4.2 Facility Points of Contact

FPOCs for facilities where biological research materials are used or stored shall:

- Know where biological materials are used, produced, stored, or handled in any way in the facility.
- Be familiar with this document and its contents and objectives.

4.3 Responsible Individuals

Responsible Individuals shall:

- Develop IWSs and Safety Plans, as needed.
- Obtain approval from appropriate institutional committees (i.e., LBOC, IBC, IRB, and IACUC). For more information, contact the chair of the respective committee or the area ES&H Team.
- Implement the controls in this document as applicable to their operations.
- Ensure that workers have applicable information and training regarding health, hazards, and communication before beginning specific tasks involving biological materials.
- Plan activities involving the safe use of biological materials.
- Perform ES&H evaluations in coordination with the ES&H Teams.
- Provide the required PPE to workers who work with biological materials.

4.4 Authorizing Individuals

Authorizing individuals:

- Are appointed by the authorizing organization to fulfill its responsibilities.

- Approve procedures or delegate approval authority.
- Authorize work once all controls have been confirmed to be implemented.
- Shall, before authorizing proposed work with biohazardous research materials, ensure that the ISM process has been followed to incorporate all necessary reviews and approvals.
- Determine which employees are required to participate in the Immunization Program of the Health Services Department.

4.5 Payroll Supervisors

Payroll supervisors shall work with the ES&H Team clinician and Industrial Hygienist to determine those individuals who shall participate in the medical surveillance program.

4.6 LLNL Biosafety Operations Committee

LBOC shall:

- Determine whether a proposed activity requires peer review, institutional review (i.e., through the IBC, IRB, or IACUC), ES&H review, and National Environmental Policy Act (NEPA) review, and inform both the Responsible Individual and the reviewing organizations of the need for review.
- Review the biological research materials, and their hazards, involved in the proposed activity.
- Make initial recommendations for training, medical surveillance, environmental protection, waste disposal, and containment levels for the proposed activity.
- Identify ethical issues associated with the proposed activity.
- Review and recommends proposed changes to the *ES&H Manual* involving biological operations.
- Address biosafety issues as requested by the Council of Biosciences and Biotechnology (CBB).
- Review any new or modified biological research operations and procedures that may impact research activities. Such activities include the intended use of any new equipment or procedures for the handling or

processing of biological materials into or from LLNL facilities, or the introduction of new biological materials into LLNL.

- Document the discussions, agreements, and commitments of each meeting in the form of minutes that are retained by the LBOC Chair and distributed to all LBOC members, the CBB Chair, and others as deemed appropriate.

4.7 LLNL Institutional Biosafety Committee

The IBC:

- Shall review and approve all LLNL research activities involving recombinant DNA and biohazardous materials. Through review, the IBC ensures that such activities and related facilities are in compliance with applicable LLNL policies and external regulations.
- Shall function to fulfill LLNL's obligations under current governmental requirements, including those dealing with recombinant DNA activities, human gene therapy techniques, biohazardous agents, and select agent transfer. To this end, the IBC shall assist Responsible Individuals in meeting their responsibilities.
- May at any time suspend an activity involving biohazardous agents or recombinant DNA that is not in compliance with LLNL policies or external regulations. Upon doing so, the IBC immediately notifies the affected Authorizing Individuals, Responsible Individuals, appropriate LLNL officers, and others as required by LLNL policies and external regulations.
- Shall approve this document as the institutional biosafety manual.
- Shall perform the additional duties defined in Section IV B-2 of the *NIH Guidelines*, which are available at the following Internet address:

<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

4.8 Health Services Department

The Health Services Department shall:

- Provide a medical surveillance program for employees who work with or who are at risk for occupational exposure to biohazardous materials.

- Provide onsite medical assessment and treatment of significant exposures and (in collaboration with the Hazards Control Department) worksite assessment to reduce the hazard of subsequent exposures.
- Maintain the medical records of employees who work with biological materials, including a record of immunization, surveillance, and post-exposure assessment and treatment.
- Provide advice, medical safety consultation, and prophylactic measures as needed.
- Serve as a required member of the IBC.

4.9 Hazards Control Department

The Hazards Control Department shall:

- Assist in the identification of hazards associated with biological materials.
- Assist employees in working safely with biological materials.
- Concur with IWSs for work involving biological research materials.
- Review HAC forms (or equivalent) for operations involving biological materials.
- Determine the need for and frequency of workplace monitoring and inform supervisors, workers, and the Health Services Department of the results.
- Work collaboratively with the Health Services Department in the post-exposure and followup programs.
- Provide general training as requested.
- Designate LLNL's biological safety officer as the subject-matter expert on biosafety.
- Works collaboratively with HSD and payroll supervisor to identify individuals requiring medical surveillance.

4.10 Biosafety Officer

The biosafety officer's duties include, but are not be limited to, the following:

- Performing periodic inspections to ensure that laboratory standards are rigorously followed.

- Reporting to LLNL and the IBC any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which he or she becomes aware, unless he or she determines that a report has already been filed by the Responsible Individual.
- Developing emergency plans for handling accidental spills and personnel contamination and for investigating laboratory accidents involving recombinant DNA research.
- Providing advice on laboratory security.
- Providing technical advice to Responsible Individuals and the IBC regarding research safety procedures.
- Represent LLNL in institutional biosafety issues involving external assessments

4.11 Environmental Protection Department

This section defines the responsibilities of the Environmental Protection Department. Contact the area environmental analyst for information regarding medical waste management, including waste characterization, storage procedures, and preparation for waste treatment or disposal. The area Hazardous Waste Management (HWM) Division field technician can assist in ensuring receipt of proper biohazardous and sharps containers, labels, and waste disposal requisition forms that need to accompany all sharps wastes and biohazardous wastes with a hazardous or radioactive component.

4.11.1 Environmental Analysts

Environmental analysts shall:

- Review the HAC forms (or equivalent) for operations involving the use of biological materials.
- Provide guidance to biohazard handlers on how to implement environmental controls and procedures and on the proper management of hazardous waste contaminated with biohazards to ensure compliance with all applicable federal, state, and local environmental requirements.
- Provide specific training to programs and divisions, as required.

4.11.2 Hazardous Waste Management Technicians

HWM technicians shall:

- Provide specific guidance to biohazard handlers on how to properly segregate, package, and label solid and liquid wastes that are contaminated with biohazardous materials.
- Coordinate the disposal of waste generated in the area.

5.0 Work Standards

5.1 Work Smart Standards

7 CFR Chapter III, Part 330, "Federal Plant Pest Regulations; General; Plant Pests; Soil; Stone, and Quarry Products; Garbage."

9 CFR Chapter I, Part 104, "Permits for Biological Products."

9 CFR 104, Subchapter E, "Viruses, Serum, Toxins, and Analogous Products, Organisms, and Vectors."

29 CFR 1910.1030, "Bloodborne Pathogens," included in 29 CFR 1910, Subpart Z, Toxic & Hazardous Substances (1910.1000 to 1910.1450 App B).

42 CFR 73, "Possession, Use, and Transfer of Select Agents for Humans."

Biosafety in Microbiological and Biomedical Laboratories, 4th Edition 1999, U.S. Dept. of HHS, Public Health Service, HHS Publication No. (CDC) 93-8395.

NIH Guidelines for Research Involving Recombinant DNA Molecules (66 FR 1146). Revised January 2001. Available at the following Internet address:

<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

Public Law, 104-32, "The Anti-Terrorism and Effective Death Penalty Act (AEDPA)," 1996.

Public Law, 107-56, "The Patriot Act of 2001."

Public Law 107-188, "Public Health and Security and Bioterrorism Preparedness and Response."

5.2 Other Requirements

7 CFR 331, "Possession of Biological Agents and Toxins" for plants.

9 CFR 121, "Possession of Biological Agents and Toxins" for animals.

42 CFR 72, "Interstate Shipment of Etiological Agents."

NIH Guidelines for Research Involving Recombinant DNA Molecules. Revised April 2002. Available at the following Internet address:

<http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html>

6.0 Resources for More Information

6.1 Contacts

See the ES&H Contact List.

6.2 Lessons Learned

For lessons learned applicable to biological materials, refer to the following Internet address:

http://www-r.llnl.gov/es_and_h/lessons/lessons.shtml

6.3 Other Sources

Hunt, D.L., 1995, "Human Immunodeficiency Virus Type 1 and Other Bloodborne Pathogens," pages 33–66. In Fleming, Richardson, Tulis, Vesley (eds), *Laboratory Safety: Principles and Practices*, 2nd Edition, American Society of Microbiology.

International Air Transport Association, Dangerous Goods Program Web site:

<http://www.iata.org/cargo/dg/index.htm>

Jahrling, Peter, 1985, "Marburg Virus, Ebola Virus, and the Arenaviruses," pages 796–804. In Lennette, Balows, Hausler, and Shadomy (eds), *Manual of Clinical Microbiology*. 4th Edition, American Society of Microbiology.

National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, 1974 (DHEW, Government Printing Office, Pub. No. NIH 75-790).

NIH Laboratory Safety Monograph, "A Supplement to the NIH Guidelines for Recombinant DNA Research," July 1978.

Swenson, P.D., 1991, "Hepatitis Viruses," pages 959–983. In Balows, Hausler, Herman, Isenberg and Shadomy (eds), *Manual of Clinical Microbiology*. 5th Edition, American Society of Microbiology.

Appendix A

Acronyms, Terms, and Definitions

Biohazardous agent	A substance that is biological in nature and usually capable of self-replication and has the capacity to produce deleterious effects on other biological organisms, particularly humans. Biohazardous agents include, but are not limited to, various viruses, prions, chlamydia, bacteria, fungi, yeast, and algae, as well as plants and animals and their products that contain any of these agents.
Biohazardous material	A biologically derived material, such as a toxin or the component of a biological agent, that presents a risk of causing illness or injury to humans, plants, and animals and that is not capable of self-replication
Biohazard	Any biological material, or a component thereof, that presents a risk of illness or injury to humans, plants, and animals.
Biological safety	A complete program of administrative controls, medical surveillance, vaccination, and containment strategies for reducing the risk of disease to employees facing potential occupational exposure to infectious agents or other biologically derived molecules. Biosafety protects workers, products, coworkers, laboratory support workers, the environment, and the general public. Used interchangeably with “biosafety.”
Biological safety cabinet (BSC)	A ventilated cabinet that serves as the primary containment device for operations involving biohazardous materials. There are three classes of biological safety cabinets (i.e., Classes 1 through 3).
Biosafety	See “Biological safety.”
Biosafety (containment) level	The level of containment required to perform biohazardous operations safely. Work practices and techniques, safety equipment, and laboratory facilities appropriate for the operations are based on the hazards imposed by the agents used and for laboratory function and activities.

Biosurety	Defined by the Department of Energy (DOE) as an integrated approach to the management and oversight of potentially hazardous biological materials and activities in the fields of recombinant DNA, genetic research, and environmental remediation. Ensures safety, emergency management, security, community relations, and environmental protection.
Blood (human)	Human blood, human blood components, and products made from human blood.
Bloodborne pathogen	A pathogenic microorganism that is present in human blood and can cause disease in humans. Such pathogens include, but are not limited to, HBV and HIV.
BSC	See "Biological safety cabinet."
BSL	Biosafety level.
CBB	LLNL Council of Biosciences and Biotechnology.
CDC	Centers for Disease Control and Prevention. An agency of the Department of Health and Human Services.
Containment	A safe method for managing infectious agents in a laboratory environment where they are handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the environment to potentially hazardous agents.
Contaminated	A term indicating the presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.
Decontamination	The use of physical or chemical means to remove, inactivate, or destroy pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal.
Disinfection	The use of antimicrobial agents on inanimate objects to destroy all organisms that could pose a potential hazard to humans or animals or compromise the integrity of an experiment.

DNA	Deoxyribonucleic acid. DNA, the nucleic acid in which the sugar is a deoxyribose, constitutes the primary genetic material of all cellular organisms and DNA viruses. DNA can be single or double stranded.
Engineered control	An engineered device or control that isolates or removes hazards from the workplace.
Etiologic agent	A disease-causing agent.
First-degree relative	A person separated from an individual by only one step in a direct hereditary line of descent or ascent, i.e., a parent, sibling, or child.
Fume hood	A ventilated work enclosure with one open side through which air flows. The height or width of the opening can be adjusted by a sash and/or door(s). Air velocity is highest at the front opening (i.e., face plane). Airflow is distributed inside the hood by means of a plenum at the back with slot-type openings. Unlike a spray booth, where air velocity is essentially constant from front to back, the velocity of air inside the hood decreases rapidly after it has passed through the face plane.
Genomic materials	Material (i.e., DNA or RNA) obtained from the genome of an organism.
HAC	Hazard Assessment and Control (form)
Handwashing facility	A facility providing an adequate supply of running potable water, soap, and single-use towels or hot air drying machines.
Hazardous material	A material that is explosive, flammable, poisonous, an irritant, or otherwise harmful and likely to cause injury or illness.
HBV	Hepatitis B virus.
HEPA	See "High-efficiency particulate air (HEPA) filter."
High-efficiency particulate air (HEPA) filter	A filter capable of trapping and retaining at least 99.97% of all monodispersed particles 0.3 microns in diameter.
HIV	Human immunodeficiency virus.

IACUC	Institutional Animal Care and Use Committee.
Institutional Biosafety Committee (IBC)	A committee for biosafety research that meets the requirements specified in Section IV B-2, "Institutional Biosafety Committee (IBC)," of the <i>NIH Guidelines</i> and that reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV B-2.
IRB	Institutional Review Board.
Laboratory-scale work	Work with substances in which the containers used for reactions, transfers, and other handling of substances are designed to be easily and safely manipulated by one person. "Laboratory scale" excludes those workplaces whose function is to produce commercial quantities of materials.
LBOC	LLNL Biosafety Operations Committee. An advisory committee.
Negative-air-flow unit	A unit that draws air into a facility to replace the air removed by exhaust systems or other facility conditions.
NIH	National Institutes of Health (an agency of the Public Health Service).
Oncogenic virus	A virus that causes cancer.
Pathogen	Any agent (usually living) capable of producing disease.
Personal protective equipment (PPE)	Specialized clothing or equipment worn by an employee for protection against a hazard. General work clothes (e.g., uniforms, pants, shirts, or blouses) not intended to function as protection against a hazard are not considered to be PPE.
PPE	See "Personal protective equipment (PPE)."
Prion	Small proteinaceous infectious particles.
Production facility	A facility engaged in industrial-scale, large-volume, or high-concentration production of microorganisms.

Recombinant DNA molecules	Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell. This definition includes molecules that result from the replication of such molecules.
Regulated biohazardous waste	Liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; or items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling, e.g., contaminated sharps, pathological waste, or microbiological wastes containing blood or other potentially infectious materials.
Research laboratory	A laboratory producing or using research laboratory-scale amounts (i.e., amounts greater than 10 liters) of biological materials or chemicals.
Responsible Individual	The individual directly responsible for an operation, activity, or group of activities. The Responsible Individual may be at any level within the organization and is formally identified by the activity's authorizing individual. In some organizations, this person is called the work supervisor. In most cases the Responsible Individual will be directing the work of others as part of the operation or activity. See Documents 2.1 and 2.2 for more information.
RG	See "Risk group."
Risk group	A system (developed by the CDC and NIH) for classifying biological agents by the degree of hazard. There are four risk groups: A higher RG number indicates a higher level of hazard.
RNA	Ribonucleic acid. RNA, the nucleic acid in which the sugar is ribose, constitutes the genetic material in the RNA viruses and in all biology is the intermediary between genetic material and the synthesis of protein.

Select agent	<p>A microorganism (e.g., virus, bacterium, fungus, or rickettsia) or toxin listed in 42 CFR 73 (Possession, Use, and Transfer of Select Agents for Humans). Select agents have been identified as potential weapons of mass destruction that can be used in bioterrorism activities. The term “select agent” also includes:</p> <ul style="list-style-type: none"> • Genetically modified microorganisms or genetic elements from organisms in Appendix A of this document that are shown to produce or encode for a factor associated with a disease. • Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins in Appendix B of 42 CFR 72 or their toxic subunits.
Sharps	Any object that can penetrate the skin, including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed metal edges (e.g., dental wires).
SOP	Standard operating procedure.
Sterilize	To use a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.
Universal precautions	An approach to infection control under which all human blood and certain human bodily fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens (see Appendix G).
Work practice control	A control that reduces the likelihood of exposure by altering the manner in which a task is performed.
Zoonotic	Readily communicable from animals to humans.

Appendix B

Biosafety Levels

This document adopts the classification system of *Biosafety in Microbiological and Biomedical Laboratories* (see Section 5.1) for BSLs. This classification system assigns BSL 1 through 4. The higher the BSL number, the higher the containment level that is required. Although BSL 3 and 4 containment levels are discussed below, work at LLNL is restricted to BSL 1 and 2. The requirements for BSL 1 and BSL 2 are summarized in Table B-1. BSL 3 controls are listed for reference purposes only, i.e., to show the next level of control.

Biosafety Level 1

BSL 1 requires the use of standard laboratory practices and equipment found in most research and teaching institutions. Such a laboratory provides areas of open bench space, with no special containment equipment, and is generally not well separated from the general traffic of the building. This type of laboratory is suitable for experiments involving RG 1 agents of no known hazard, or minimal potential hazard, to laboratory personnel or the environment.

Biosafety Level 2

BSL 2 requires the use of standard laboratory practices and equipment designed for experiments with agents of moderate hazard to personnel and the environment. BSL 2 differs from BSL 1 mainly in providing areas of containment (e.g., a BSC) to carry out certain laboratory experiments and operations in which aerosols could be created. Examples of experiments requiring BSL 2 containment include:

- Recombinant DNA work involving nonexempt microorganisms (as defined in the *NIH Guidelines*), such as *Salmonella* and *Pseudomonas fluorescens*.
- Work with many bacterial species, including all species and serotypes of *Salmonella*.
- Work with many viral agents, such as measles viruses, rhinoviruses, mumps, and polioviruses.
- Work with low concentrations or small volumes of low- or moderate-risk oncogenic viruses, such as the Epstein-Barr virus or feline leukemia virus.
- Work with tissues or bodily fluids from human or primates.

- Work with viable human tumor cells or tumor cell lines.

Table B-1. Biological research laboratory containment levels of the Centers for Disease Control and National Institutes of Health.

		BSL 1	BSL 2	BSL 3
A	Hazard Levels	Basic/Low	Moderate Risk	Moderate to High Risk
	(Examples)	Undergraduate secondary training	Clinical or diagnostic laboratory	Clinical, diagnostic, production, teaching, or research settings
B	Standard Microbiological Practices			
1	Public access while experiments are in progress	Limited	Limited/ restricted	Limited/ restricted or Controlled
2	Decontamination	Daily	Daily and after spills	Daily and after spills
3	Regulated waste decontamination	Before disposal	Before disposal	Before disposal
4	Pipetting (No mouth pipetting)	Mechanical devices	Mechanical devices	Mechanical devices
5	Eating, drinking, smoking, applying cosmetics, and handling contact lenses	Not permitted	Not permitted	Not permitted
6	Aerosol minimization	Recommended	Recommended	Recommended
7	Food Storage	Outside of work area	Outside of work area	Outside of work area
8	Handwashing	Frequently and before leaving the laboratory	Frequently and before leaving the laboratory	Frequently and before leaving the laboratory
9	Aerosol production	Prohibited in open lab bench	BSC, centrifuge safety cups	BSC, centrifuge safety cups
10	Sharps protocol	Required	Required	Required
11	Lab coats not worn outside the laboratory	Recommended	Required (front-buttoned coats)	Required (wrap-around coats)
12	Face protection	Required	Required	Required
C	Special Practices			
1	Onsite autoclave	Not required in laboratory	Not required, but available onsite	Required onsite
2	Rodent control program	Required	Required	Required
3	Transport of infectious materials for decontamination purposes	In labeled, durable, leakproof container with closeable lid	In labeled, durable, leakproof container with closeable lid	In labeled, durable, leakproof container with closeable lid

4	Animals in work area but not being used in lab experiments	Not permitted	Not permitted	Not permitted
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Table B-1. Biological research laboratory containment levels of the Centers for Disease Control and National Institutes of Health. (cont'd.)

		BSL 1	BSL 2	BSL 3
D	Safety Equipment			
1	Biological safety cabinet (BSC) or other containment systems	Recommended	Class II BSC required for use of aerosol-generating equipment or high concentrations of agents, for animal inoculation, or for harvesting of infected animal tissue or eggs	Class II BSC required for use of aerosol-generating equipment or high concentrations of agents, for animal inoculation, or for harvesting of infected animal tissue or eggs
2	BSC testing	When new, after moving, annually thereafter	When new, after moving, annually thereafter	When new, after moving, annually thereafter
3	Personal protective equipment			
	a. Face protection	Recommended	Required whenever splashes are anticipated	Required whenever splashes are anticipated
	b. Gloving	Recommended. Required for workers with broken skin	Required when handling infectious materials. Double gloving suggested	Required when handling infectious materials. Double gloving suggested
E	Laboratory facilities			
1	Ventilation	Not required	Negative pressure	Negative pressure with audible alarm system. No air recirculation to other parts of the building
2	Open windows	Permitted, but fly screens required	Permitted, but fly screens required	Prohibited. Closed and sealed windows required
3	Doors	Required	Lockable door required for certain agents	Double doors

4	Posted warning sign	Recommended with agent and PI identified	Required with agent and PI identified	Required with agent and PI identified
5	Lab separated from general public	No, but access control required	Yes. Access control also required	Yes. Access control also required

Table B-1. Biological research laboratory containment levels of the Centers for Disease Control and National Institutes of Health. (cont'd.)

		BSL 1	BSL 2	BSL 3
6	Handwashing facility	Required	Required	Required
7	Shower facilities	Not required	Not required	Recommended
8	Chemical shower	Not required	Not required	Recommended
9	HEPA vacuum lines	Recommended	Recommended	Required
F	Technical training	Required	Required	Required
G	Medical surveillance	Recommended	Required when appropriate	Required when appropriate

Reference: Centers for Disease Control and National Institutes of Health, *Biosafety in Microbiological and Biomedical Laboratories*, 4th edition, (U.S. Government Printing Office, Washington, DC, 1999, DHHS Pub. No. 93-8395).

Operations that involve large volumes or high concentrations of certain RG 2 agents, such as the hepatitis viruses or Epstein-Barr virus, should be carried out at BSL 3. See *Biosafety in Microbiological and Biomedical Laboratories* for guidance.

Biosafety Level 2 Safe Work Practices for Laboratory Staff

Safe work practices for laboratory staff include, but are not limited to, the following:

- Follow the universal precautions (see Appendix G) at all times.
- Protective eyewear and face shield shall be worn for procedures that commonly result in the generation of droplets or the splashing of blood or other bodily fluids.
- Laboratory coats shall be worn during all laboratory procedures. Additional protection (e.g., gowns and aprons) should be worn during procedures in which the splashing of blood or other bodily fluids can be reasonably anticipated.
- Gloves shall be worn during all procedures that involve the handling of items containing or contaminated with blood and in areas where items (e.g., benches) could be contaminated with potentially infectious materials.

- A glove that is torn shall be removed and replaced promptly.
- Gloves shall be changed and hands washed after completion of specimen processing.
- All specimens of blood and bodily fluids shall be put in a well-constructed container with a secure lid to prevent leaking during transport.
- Care shall be taken when collecting each specimen to avoid contaminating the outside of the container or any form (or other paperwork) accompanying the specimen.
- For routine procedures, such as histological and pathological studies or microbiological culturing, a BSC is highly recommended.
- BSCs (or equivalent protective ventilation controls) shall always be used for procedures that have a high potential for generating aerosols.
- Mechanical pipetting devices shall be used for manipulating all liquids in the laboratory. Mouth pipetting is prohibited.
- Needles and syringes should be used only when no alternatives exist. Employees should pay attention to their hands whenever handling needles and syringes.
- Laboratory work surfaces shall be decontaminated with an appropriate chemical germicide after a spill of blood or other bodily fluids and when work activities are completed.
- Equipment contaminated with blood or other potentially infectious materials *shall* (and equipment contaminated with any other biological material *should*) be cleaned with a mild bleach solution (i.e., 1:10 to 1:100 dilution of household bleach) or with an appropriate chemical germicide immediately after completion of laboratory procedures. Contaminated equipment should never be stored without the appropriate biohazard label. An acceptable alternative is autoclaving.

[The cleaning of chemically or radiologically contaminated equipment is covered in other *ES&H Manual* documents, e.g., those in Part 14 (Chemical) and Part 20 (Ionizing Radiation/Nonionizing Radiation).]

- All laboratory staff shall wash their hands after completing laboratory activities and shall remove protective clothing before leaving the laboratory.
- Any personal clothing that is contaminated with blood or other bodily fluids during collection procedures shall be removed immediately (or as

soon as possible) to prevent further contamination and separated from other clothing until properly laundered.

In accordance with *Biosafety in Microbiological and Biomedical Laboratories* and the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, certain work with RG 3 agents in BSL 2 containment facilities may be allowed after approval of the program, the IBC, and the ES&H Team has been obtained. Such work requires additional controls.

Biosafety Level 3

No work classified at BSL 3 is currently authorized at LLNL. Agents in this category include those which may cause serious or potentially lethal disease as a result of inhalation, viable organisms, or recombinant DNA agents classified as RG 3 agents (including *Mycobacterium tuberculosis*, *Histoplasma capsulatum*, most arboviruses, and the rabies virus), and moderate-risk oncogenic viruses involving high concentrations or large volumes.

Biosafety Level 4

No work classified at BSL 4 is authorized at LLNL. Such work would involve dangerous and exotic agents that present a high risk of life-threatening disease. Examples of such agents are the Lassa fever virus, Kyasanur Forest disease virus, Marburg virus, wild strains of yellow fever virus, recombinant DNA agents that carry genes for the biosynthesis of molecules highly toxic for vertebrates, and recombinant DNA agents that carry genes for drug resistance in microorganisms not known to acquire resistance naturally when acquisition could compromise the use of the drug to control disease. Stringent laboratory practices are required, and maximum containment equipment and facilities are required.

Appendix C

Classification of Human Etiologic Agents

This appendix (excerpted from Appendix B of the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, January 2001) includes biological agents known to infect humans, as well as selected animal agents that may pose theoretical risks if inoculated into humans. Included are lists of representative genera and species known to be pathogenic; mutated, recombined, and nonpathogenic species and strains are not considered. Noninfectious life cycle stages of parasites are excluded.

RISK GROUP (RG) 1 AGENTS	
Bacillus subtilis or Bacillus licheniformis (Asporogenic)	
Bacillus subtilis or Bacillus licheniformis host-vector systems, exceptions	
Escherichia coli-K12	
Host-vector systems, exceptions	
Adeno-associated virus (AAV) Types 1 through 4, recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus.	
Those agents not listed in RG 2, 3, and 4 are not automatically or implicitly classified in RG 1; a risk assessment shall be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.	
RG 2 AGENTS	
Bacterial Agents including Chlamydia	
Acinetobacter baumannii (formerly Acinetobacter calcoaceticus)	
Actinobacillus	
Actinomyces pyogenes (formerly Corynebacterium pyogenes)	
Aeromonas hydrophila	
Amycolata autotrophica	
Archaeobacterium haemolyticum (formerly Corynebacterium haemolyticum)	
Arizona hinshawii (all serotypes)	
Bacillus anthracis	
Bartonella henselae, B. quintana, B. vinsonii	
Bordetella including B pertussis	
Borrelia recurrentis	
B. burgdorferi	
Burkholderia (formerly Pseudomonas species), except those listed in this Appendix as RG 3	
Campylobacter coli	
Campylobacter fetus	

RG 2 AGENTS (cont'd.)
Bacterial Agents including Chlamydia
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis
Chlamydia pneumoniae
Clostridium botulinum
Clostridium chauvoei
Clostridium haemolyticum
Clostridium histolyticum
Clostridium novyi
Clostridium septicum
Clostridium tetani
Corynebacterium diphtheriae
Corynebacterium pseudotuberculosis
Corynebacterium renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix rhusiopathiae
Escherichia coli (all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen), including E. coli O157:H7
Haemophilus ducreyi
Haemophilus influenzae
Helicobacter pylori
Klebsiella – all species except K. oxytoca (RG 1)
Legionella including L. pneumophila
Leptospira interrogans (all serotypes)
Listeria
Moraxella
Mycobacterium (except those listed in this Appendix as RG 3, including M. avium complex)
M. asiaticum
M. bovis (BCG vaccine strain)
M. chelonae
M. fortuitum
M. kansasii
M. leprae

M. malmoense

RG 2 AGENTS (cont'd.)
Bacterial Agents including Chlamydia
M. marinum
M. paratuberculosis
M. scrofulaceum
M. simia
M. szulgai
M. ulcerans
M. xenopi
Mycoplasma, except M. mycoides and M. agalactiae, which are restricted animal pathogens
Neisseria gonorrhoeae
Neisseria meningitidis
Nocardia asteroides
Nocardia brasiliensis
Nocardia otitidiscaviarum
Nocardia transvalensis
Rhodococcus equi
Salmonella, including: <ul style="list-style-type: none"> S. arizonae S. choleraesuis S. enteritidis S. gallinarum-pullorum S. meleagridis S. paratyphi-A, B, C S. typhi S. typhimurium
Shigella, including: <ul style="list-style-type: none"> S. boydii S. dysenteriae-type 1 S. flexneri S. sonnei
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae

Streptococcus pyogenes
Treponema carateum

RG 2 AGENTS (cont'd.)
Bacterial Agents including Chlamydia
Treponema pallidum
Vibrio cholerae
Vibrio parahemolyticus
Vibrio vulnificus
Yersinia enterocolitica
Fungal Agents – RG 2
Blastomyces dermatitidis
Cladosporium bantianum
C. (Xylohypha) trichoides
Cryptococcus neoformans
Dactylaria galopava (ochroconis gallopavum)
Epidermophyton
Exophiala (Wangiella) dermatitidis
Fonsecaea pedrosoi
Microsporum
Paracoccidioides braziliensis
Penicillium marneffeii
Sporothrix schenck
Trichophyton
Parasitic Agents – RG 2
Ancylostoma human hookworms, including A. duodenale A. ceylanicum
Ascaris, including Ascaris lumbricoides suum
Babesia, including: B. divergens B. microti
Brugia filaria worms, including: B. malayi B. timori
Coccidia

Cryptosporidium, including <i>C. parvum</i>
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RG 2 AGENTS (cont'd.)
Parasitic Agents – RG 2
Cysticercus cellulosae (hydatid cyst, larva of <i>T. solium</i>)
Echinococcus including: <i>E. granulosus</i> <i>E. multilocularis</i> <i>E. vogeli</i>
Endamoeba histolytica
Enterobius
Fasciola including: <i>F. gigantica</i> <i>F. hepatica</i>
Giardia, including <i>G. lamblia</i>
Heterophyes
Hymenolepis including: <i>H. diminuta</i> <i>H. nana</i>
Isospora
Leishmania, including: <i>L. braziliensis</i> <i>L. donovani</i> <i>L. ethiopia</i> <i>L. major</i> <i>L. mexicana</i> <i>L. peruviana</i> <i>L. tropica</i>
Loa loa filaria worms
Microsporidium
Naegleria fowleri
Necator human hookworms, including <i>N. americanus</i>
Onchocerca filaria worms, including <i>O. volvulus</i>
Plasmodium, including simian species
<i>P. cynomolgi</i>
<i>P. falciparum</i>

P. malariae
P. ovale
P. vivax

RG 2 AGENTS (cont'd.)
Parasitic Agents – RG 2
Sarcocystis, including <i>S. sui hominis</i>
Schistosoma, including: <ul style="list-style-type: none"> <i>S. haematobium</i> <i>S. intercalatum</i> <i>S. japonicum</i> <i>S. mansoni</i> <i>S. mekongi</i>
Strongyloides, including <i>S. stercoralis</i>
Taenia solium
Toxocara, including <i>T. canis</i>
Toxoplasma, including <i>T. gondii</i>
Trichinella spiralis
Trypanosoma, including: <ul style="list-style-type: none"> <i>T. brucei bruce</i> <i>T. brucei gambiense</i> <i>T. brucei rhodesiense</i> <i>T. cruzi</i>
Wuchereria bancrofti filaria worms
RG 2 VIRUSES
Adenoviruses (human, all types)
Alphaviruses (Togaviruses) – Group A Arboviruses <ul style="list-style-type: none"> – Eastern equine encephalomyelitis virus – Venezuelan equine encephalomyelitis vaccine strain TC-83 – Western equine encephalomyelitis virus
Arenaviruses <ul style="list-style-type: none"> – Lymphocytic choriomeningitis virus (non-neurotropic strains) – Tacaribe virus complex – Other viruses

Bunyaviruses <ul style="list-style-type: none"> – Bunyamwera virus – Rift Valley fever virus vaccine strain MP-12 – Other viruses (See Ref.)
Calciviruses
Coronaviruses

RG 2 VIRUSES (cont'd.)
Flaviviruses (Togaviruses) – Group B Arboviruses <ul style="list-style-type: none"> – Dengue virus serotypes 1, 2, 3, and 4 – Yellow fever virus vaccine strain 17D – Other viruses (See Ref.)
Hepatitis A, B, C, D, and E viruses
Herpesviruses [except Herpesvirus simiae (Monkey B virus), which is in RG4] <ul style="list-style-type: none"> – Cytomegalovirus – Epstein-Barr virus – Herpes simplex types 1 and 2 – Herpes zoster – Human herpesvirus types 6 and 7
Orthomyxoviruses <ul style="list-style-type: none"> – Influenza viruses types A, B, and C – Other tick-borne orthomyxoviruses (See Ref.)
Papovaviruses <ul style="list-style-type: none"> – All human papilloma viruses
Paramyxoviruses <ul style="list-style-type: none"> – Newcastle disease virus – Measles virus – Mumps virus – Parainfluenza viruses types 1, 2, 3, and 4 – Respiratory syncytial virus
Parvoviruses <ul style="list-style-type: none"> – Human parvovirus (B19)

Picornaviruses <ul style="list-style-type: none"> – Coxsackie viruses types A and B – Echoviruses (all types) – Polioviruses (all types, wild and attenuated) – Rhinoviruses (all types)
Poxviruses – all types except Monkey-pox virus (see RG 3)
Viruses and Prions and restricted poxviruses, including: <ul style="list-style-type: none"> Alastrim Smallpox Whitepox
Reoviruses – all types except coltivirus, human rotavirus, and orbivirus (Colorado tick virus)
Rhaboviruses, including: <ul style="list-style-type: none"> Rabies virus (all strains) Vesicular stomatitis virus (laboratory-adapted strains, including VSV-Indian, San Juan, and Glasgow)
RG 2 VIRUSES (cont'd.)
Togaviruses (see Alphaviruses and Flaviviruses) <ul style="list-style-type: none"> – Rubivirus (rubella)
RG 3
Bacterial Agents, including Rickettsia
Bartonella
Brucella, including: <ul style="list-style-type: none"> B. abortus B. canis B. suis
Burkholderia (Pseudomonas) mallei
B. pseudomallei <ul style="list-style-type: none"> – Coxiella burnetii – Francisella tularensis – Mycobacterium bovis (except BCG strain) RG 2
M. tuberculosis
Pasteurella multocida type B (“buffalo” and other virulent strains)
Rickettsia akari <ul style="list-style-type: none"> R. australis R. canada R. conorii R. prowazekii R. rickettsii R. siberica

R. tsutsugamushi
R. typhi (R. mooseri)
Yersinia pestis
Fungal Agents – RG 3
Coccidioides immitis (sporulating cultures and contaminated soil)
Histoplasma capsulatum
Histoplasma capsulatum var. duboisii
Parasitic Agents – RG 3
None

RG 3 (cont'd.)
Viruses and Prions – RG 3
Alphaviruses (Togaviruses) – Group A Arboviruses
<ul style="list-style-type: none"> – Semliki Forest viruses – St. Louis encephalitis virus – Venezuelan equine encephalomyelitis virus (except the vaccine strain TC-83) – Other viruses
Arenaviruses
<ul style="list-style-type: none"> – Flexal – Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
Bunyaviruses
<ul style="list-style-type: none"> – Hantaviruses
Hantaan virus
<ul style="list-style-type: none"> – Rift Valley fever virus
Flaviviruses (togaviruses) Group B arboviruses
<ul style="list-style-type: none"> – Japanese encephalitis virus – Yellow fever virus – Other viruses
Poxviruses
Monkeypox virus
Prions
<ul style="list-style-type: none"> – Transmissible spongiform encephalopathies (TSE) agents (Creutzfeldt-Jacob disease and kuru agents)
Retroviruses
<ul style="list-style-type: none"> – Human immunodeficiency virus (HIV) types 1 and 2 – Human T cell lymphotropic virus (HTLV) types 1 and 2

Simian immunodeficiency virus (SIV)
Rhabdoviruses
– Vesicular stomatitis virus
RG 4 AGENTS
Bacterial Agents – RG 4
None
Fungal Agents – RG 4
None
Parasitic Agents – RG 4
None

RG 4 AGENTS (cont'd.)
Viral Agents – RG 4
Arenaviruses
– Guaranito virus
– Lassa virus
– Junin virus
– Machupo virus
– Sabia
Bunyaviruses (Nairovirus)
– Crimean-Congo hemorrhagic fever virus
Filoviruses
– Ebola virus
– Marburg virus
Flaviruses (Togaviruses) – Group B arboviruses
Tick-borne encephalitis virus complex, including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses
Herpesviruses (alpha)
– Herpesvirus simiae (Herpes B or Monkey B virus)
Paramyxoviruses
– Equine morbillivirus
– Hemorrhagic fever agents and viruses as yet undefined
Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans but are commonly used in laboratory experimental work. A containment level appropriate for RG 1 human agents is recommended for their use. For agents that are infectious to human cells (e.g., amphotropic and xenotropic strains of murine leukemia virus), a containment level appropriate for RG 2 human agents is recommended.

Baculoviruses

Herpesviruses

- Herpesvirus atele

Herpesvirus saimiri

- Marek's disease virus
- Murine cytomegalovirus

Papovaviruses

- Bovine papilloma virus
- Polyoma virus
- Shope papilloma virus
- Simian virus 40 (SV40)

Animal Viral Etiologic Agents in Common Use (cont'd.)

Retroviruses

- Avian leukosis virus
- Avian sarcoma virus
- Bovine leukemia virus
- Feline leukemia virus
- Feline sarcoma virus
- Gibbon leukemia virus
- Mason-Pfizer monkey virus
- Mouse mammary tumor virus
- Murine leukemia virus
- Murine sarcoma virus
- Rat leukemia virus

Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered under BSL 1 containment.

Appendix D

Select Agents

This list is taken from 42 CFR 72, Appendix A, "Select Agents."

Bacteria

Bacillus anthracis
Brucella abortus, *B. melitensis*, *B. suis*
Burkholderia (Pseudomonas) mallei
Burkholderia (Pseudomonas) pseudomallei
Clostridium botulinum
Yersinia pestis

Fungi

Coccidioides immitis

Rickettsiae

Coxiella burnetii
Rickettsia prowazekii
Rickettsia rickettsii

Toxins

Abrin
Aflatoxins
Botulinum toxins
Clostridium perfringens epsilon toxin
Conotoxins
Diacetoxyscirpenol
Ricin
Saxitoxin
Shigatoxin
Staphylococcal enterotoxins
Tetrodotoxin
T-2 toxin

Viruses

Crimean-Congo hemorrhagic fever virus
Eastern equine encephalitis virus
Ebola viruses
Equine morbillivirus
Francisella tularensis
Lassa fever virus
Marburg virus
Rift Valley fever virus
South American hemorrhagic fever viruses (i.e., Junin, Machupo, Sabia, Flexal, Guanarito)
Tick-borne encephalitis complex viruses
Variola major virus (i.e., smallpox virus)
Venezuelan equine encephalitis virus
Viruses causing hantavirus pulmonary syndrome
Yellow fever virus

Recombinant organisms/molecules

Genetically modified microorganisms or genetic elements from organisms listed in this appendix and shown to produce or encode for a factor associated with a disease.

Genetically modified microorganisms or genetic elements that contain nucleic acid sequences coding for any of the toxins listed in this appendix or the toxic subunits of such toxins.

Other restrictions

The deliberate transfer of a drug resistance trait to a microorganism that is listed in this appendix and that is not known to acquire the trait naturally is prohibited by the *NIH Guidelines for Research Involving Recombinant DNA Molecules* if such acquisition could compromise the use of the drug to control these disease agents in humans or veterinary medicine.

Appendix E

Priority Categories Used for Select Agents by the Centers for Disease Control and Prevention

This appendix lists the agents in each of the three categories (A, B, and C) that the CDC uses to classify the select agents (see Appendix D), as well as the agents in each category. The categories indicate priority in public health preparedness efforts, with Category A having the highest priority.

Category A – Highest-Priority Agents

Agents in this category:

- Can be easily disseminated or transmitted from person to person.
- Can cause high mortality, with potential for major public health impact.
- Could cause public health panic and social disruption.
- Require special action for public preparedness.

List of Category A agents:

Arenaviruses (Lassa and Junin)

Bacillus anthracis

Clostridium botulinum toxin (botulinum)

Filoviruses (Ebola and Marburg hemorrhagic fever)

Francisella tularensis (tularemia)

Variola major (i.e., smallpox) virus

Yersinia pestis

Category B – Second Highest-Priority Agents

Agents in this category:

- Are moderately easy to disseminate.
- Cause moderate morbidity.
- Require specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance.

List of Category B agents:

Alphaviruses (Venezuelan, eastern, and western equine encephalomyelitis viruses)

Brucella species (Brucellosis)

Burkholderia mallei (Glanders)

Coxiella burnetti (Q-fever)

Epsilon toxin of *Clostridium perfringens*

Ricin toxin (from the castor bean)

Staphylococcus enterotoxin B (SEB)

A subset list including, but not limited to, food or water-borne agents (i.e., *Salmonella* species, *Shigella dysenteriae*, *E. coli* O157:H7, *Vibrio cholerae*, and *Cryptosporidium parvum*)

Category C – Third Highest-Priority Agents

This category contains emerging pathogens that could be engineered for mass dissemination because of their availability, ease of production, high mortality, and major health impact.

List of Category C agents:

Hantaviruses

Multi-drug resistant tuberculosis

Nipah virus

Tickborne hemorrhagic fever viruses

Yellow fever virus

Appendix F

USDA-Regulated Materials

This appendix lists some of the materials regulated by the USDA. Approval from the USDA is required for their use, handling, transport, or storage. This list is not exhaustive; for more information, refer to the following Internet address:

<http://www.aphis.usda.gov/>

F.1 Animal Pathogens

This is a list of animal pathogens of particularly high consequence to livestock.

African horse sickness virus	Malignant catarrhal fever virus
African swine fever	Menangle virus
Akabane virus	<i>Mycoplasma capricolum</i> , mycoplasma strain F-38, <i>M. mycoides capri</i> (contagious caprine pleuropneumonia agent)
Avian influenza virus (highly pathogenic)	<i>Mycoplasma mycoides mycoides</i> (contagious bovine pleuropneumonia agent)
Blue tongue virus (exotic)	Newcastle disease virus (exotic)
Bovine spongiform encephalopathy agent	Peste des petits ruminants virus
Camel pox virus	Rinderpest virus
Classical swine fever	Sheep pox virus
<i>Cowdria ruminantium</i> (heartwater)	Swine vesicular disease virus
Foot and mouth disease virus	Vesicular stomatitis virus
Goat pox virus	
Japanese encephalitis virus	
Lumpy skin disease virus	

F.2 Plant Pathogens

The following is a list of plant pathogens regulated by the State of California and was taken from the following USDA website:

<http://www.aphis.usda.gov/ppq/permits/plantpest/pathogen.html>

F.2.1 Bacteria (by Scientific Name)

Agrobacterium radiobacter, *Agrobacterium rubi*, *Agrobacterium tumefaciens*, *Agrobacterium vitis*, *Burkholderia andropogonis*, *Burkholderia caryophylli*, *Burkholderia cepacia*, *Burkholderia cichorii*, *Burkholderia corrugata*, *Burkholderia gladioli* pv. *gladioli*, *Clavibacter michiganensis* subsp. *insidiosus*, *Clavibacter michiganensis* subsp. *michiganensis*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Erwinia amylovora*, *Erwinia carotovora* subsp. *atroseptica*, *Erwinia carotovora* subsp. *carotovora*, *Erwinia chrysanthemi*, *Erwinia chrysanthemi* pv. *chrysanthemi*, *Erwinia chrysanthemi* pv. *dieffenbachiae*, *Erwinia chrysanthemi* pv. *zeae*, *Erwinia tracheiphila*, *Pantoea stewartii* subsp. *stewartii*, *Pseudomonas syringae* pv. *apii*, *Pseudomonas syringae* pv. *atrofaciens*, *Pseudomonas syringae* pv. *coronafaciens*, *Pseudomonas syringae* pv. *glycinea*, *Pseudomonas syringae* pv. *lachrymans*, *Pseudomonas syringae* pv. *mori*, *Pseudomonas syringae* pv. *papulans*, *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae* pv. *pisi*, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *tabaci*, *Pseudomonas syringae* pv. *tomato1*, *Ralstonia solanacearum2*, *Rhodococcus fascians*, *Spiroplasma citri*, *Streptomyces scabies*, *Xanthomonas campestris* pv. *armoraciae*, *Xanthomonas campestris* pv. *campestris*, *Xanthomonas campestris* pv. *carotae*, *Xanthomonas campestris* pv. *cucurbitae*, *Xanthomonas campestris* pv. *hederiae*, *Xanthomonas campestris* pv. *juglandis*, *Xanthomonas campestris* pv. *papavericola*, *Xanthomonas campestris* pv. *pelargonii*, *Xanthomonas campestris* pv. *pruni*, *Xanthomonas campestris* pv. *raphani*, *Xanthomonas campestris* pv. *vitians*, *Xanthomonas campestris* pv. *zinniae*, *Xanthomonas fragariae*, *Xanthomonas phaseoli* pv. *alfalfae*, *Xanthomonas phaseoli* pv. *begoniae*, *Xanthomonas phaseoli* pv. *glycines*, *Xanthomonas phaseoli* pv. *phaseoli*, *Xanthomonas translucens* pv. *translucens*, *Xanthomonas vesicatoria*.

F.2.2 Fungi (by Scientific Name)

CHYTRIDIOMYCETES

Physoderma maydis

OOMYCETES

Albugo candida, *Peronospora sojae*, *Peronospora trifoliorum*, *Peronospora viticola*, *Phytophthora cactorum*, *Phytophthora capsici*, *Phytophthora cinnamomi*, *Phytophthora citricola*, *Phytophthora fragariae*, *Phytophthora infestans*, *Phytophthora megasperma*, *Phytophthora megasperma* f.sp. *medicaginis*, *Phytophthora rubi* s.sp. *fragariae*, *Phytophthora sojae*, *Plasmodiophora brassicae*, *Pythium aphanidermatum*, *Pythium arrhenomanes*, *Pythium graminicola*, *Pythium irregulare*, *Pythium ultimum*, *Sclerophthora macrospora*.

ASCOMYCETES

Apiosporina morbosa (black knot), *Botryosphaeria obtusa*, *Botryosphaeria ribis* (B. *dothidea*, B. *berengeriana*), *Claviceps purpurea*, *Cymadothea trifolii* (sooty blotch), *Diaporthe phaseolorum*,

Gaeumannomyces graminis, *Gibberella zeae*, *Glomerella cingulata*, *Leptosphaerulina trifolii*, *Monilinia fructicola* (*Sclerotinia fructicola*), *Nectria cinnabarina*, *Ophiostoma ulmi* (*Ceratocystis ulmi*), *Pseudopeziza medicaginis*, *Pseudopeziza trifolii*, *Sclerotinia sclerotiorum* (*Whetzelinia sclerotiorum*), *Sclerotinia trifoliorum*, *Valsa ambiens*, *Venturia inaequalis* (apple scab), *Xylaria polymorpha*.

Powdery Mildews

Erysiphe graminis, *Microsphaera vaccinii* (on Ericaceae), *Podosphaera clandestina* (on Rosaceae), *Sphaerotheca* Asteraceae, Cucurbitaceae, Cucurbitaceae, Scrophulariaceae), *Sphaerotheca macularis* (on hops and strawberry), *Uniclinula viticola*.

Coelomycetes

Colletotrichum acutatum, *Colletotrichum coccodes*, *Colletotrichum destructivum*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Colletotrichum graminicola*, *Colletotrichum trifolii*, *Macrophoma phaseolina* (*Macrophoma phaseolina*, *M. phaseoli*, *Botryodiplodia phaseoli*), *Phoma medicaginis*, *Phomopsis juniperovora*, *Phomopsis sojae*, *Phomopsis viticola*, *Septoria rubi*, *Septoria tritici*, *Sphaeropsis sapinea* (*Diplodia pinea*), *Stagonospora nodorum* (*Septoria nodorum*), *Stenocarpelia maydis* (*Diplodia zeae*, *D. zeae-maydis*).

Hyphomycetes

Alternaria alternata, *Alternaria solani*, *Bipolaris maydis* (*Heminthosporium maydis*, *Drechslera maydis*), *Bipolaris sorokiniana* (*Helminthosporium sorokiniana*, *Drechslera sorokiniana*), *Bipolaris victoriae* (*Helminthosporium victoriae*, *Drechslera victoriae*), *Botrytis cinerea*.

Cercospora medicaginis, *Cercospora zeae-maydis*, *Cladosporium herbarum*, *Drechslera avenae* (on oats, other grasses), *Drechslera graminea* (on barley, other grasses), *Drechslera poae* (on grasses), *Drechslera teres* (on barley, other grasses), *Drechslera tritici-repentis* (on cereals, other grasses), *Exserohilum turcicum* (*Helminthosporium turcicum*, *Bipolaris turcicum*), *Fusarium acuminatum*, *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium equiseti*, *Fusarium graminearum*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium oxysporum*, *Fusarium roseum*, *Fusarium solani*, *Penicillium expansum*, *Rhynchosporium secalis*, *Thielaviopsis basicola*, *Verticillium albo-atrum*, *Verticillium dahliae*.

HEMIASCOMYCETES

Taphrina caerulescens (leaf blister on oak, *Ostrya*, *Rhus*), *Taphrina communis* (plum pocket on *Prunus*), *Taphrina deformans* (peach leaf curl).

BASIDIOMYCETES

Wood rotters and root-collar rotters

Armillaria mellea, *Ceratobasidium cerealea*, *Daedaleopsis confragosa* (*Daedalea confragosa*), *Ganoderma applanatum* (*Fomes applanatus*), *Ganoderma lucidum*, *Hirschioporus pargamenus* (*Trichaptum biformis*, *Polyporus pargamenus*), *Laetiporus sulphureus* (*Polyporus sulphureus*), *Phellinus gilvus*, *Phellinus robiniae*, *Schizophyllum commune*, *Stereum ostrea*, *Trametes versicolor* (*Polyporus versicolor*, *Coriolus versicolor*).

Rusts

Gymnosporangium clavipes (cedar-quince rust), *Gymnosporangium globosum* (cedar-hawthorn rust), *Gymnosporangium juniperi-virginianae* (cedar-apple rust), *Puccinia coronata* (on Rhamnaceae, Eleganaceae/Poaceae), *Puccinia graminis* (on Berberis/Poaceae), *Puccinia recondita* (on Ranunculaceae/Poaceae), *Pucciniastrum americanum* (late leaf rust on raspberry).

Smuts

Tilletia caries (*Tilletia tritici*), *Tilletia laevis* (*Tilletia foetida*), *Ustilago avenae*, *Ustilago hordei*, *Ustilago tritici*, *Ustilago zeae*.

Other Basidiomycetes

Rhizoctonia solani (*Thanatephorus cucumeris*), *Sclerotium rolfsii*.

F.2.3 Viruses (Regulated by the State of California)

Alfalfa mosaic, barley yellow dwarf, bean common mosaic, bean yellow mosaic, beet curly top, beet mosaic, cactus virus X, camellia yellow mottle, carnation mottle, cauliflower mosaic, chrysanthemum mosaic, chrysanthemum virus B, cucumber mosaic, cymbidium mosaic, dasheen mosaic, fig mosaic, impatiens necrotic spot, lettuce big vein, lettuce mosaic, lily symptomless, maize dwarf mosaic, odontoglossum ringspot, papaya ringspot, pepper mottle, plum line pattern, potato leafroll, potato virus S, potato virus X, potato virus Y, prune dwarf, prunus necrotic ringspot, squash mosaic, sugarcane mosaic, tobacco etch, tomato mosaic, tomato spotted wilt, turnip mosaic, watermelon mosaic virus 2, zucchini yellow mosaic.

Appendix G

Universal Precautions

Universal precautions shall be followed to prevent contact with blood and other potentially infectious materials and thereby reduce the risk of occupational exposure. Universal precautions are an approach to infection control in which all human blood and certain human bodily fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens. The following precautions are advocated by CDC for healthcare workers:

- a. All healthcare and laboratory employees are to use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or bodily fluids is anticipated.
- b. Gloves shall be worn when touching blood, bodily fluids, mucous membranes, or non-intact skin.
- c. Gloves shall be worn when handling items or surfaces contaminated with blood or bodily fluids.
- d. Gloves shall be worn while performing venipuncture and other vascular access procedures.
- e. Gloves shall be changed after contact with each patient.
- f. During procedures that are likely to generate droplets of blood or other bodily fluids, a mask and protective eyewear (or a face shield) should be worn to prevent exposure of the mucous membranes of the mouth, nose, and eyes.
- g. Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other bodily fluids.
- h. Hands and other skin surfaces should be washed immediately and thoroughly with water and antiseptic cleanser if contaminated with blood or other bodily fluids.
- i. Hands should be immediately washed after gloves are removed.
- j. Employee shall take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments or devices during or after medical procedures, when cleaning instruments, and during disposal of used needles.
- k. To prevent needle-stick injuries, needles should not be recapped, bent or broken by hand, removed from disposable syringes, or otherwise manipulated by hand.

- l. After use, disposable syringes, needles, scalpels blades, and other sharp items shall be placed in puncture-resistant containers for disposal. These containers should be as close as practical to the area where disposable sharps are used.
- m. Laboratory employees who have exudative lesions or weeping dermatitis shall refrain from handling clinical samples or specimens and contaminated equipment until the condition is resolved.
- n. Pregnant employees should review safe work procedures with supervisors and the ES&H Team.
- o. Regulated waste shall be managed as specified in Document 36.1.

Appendix H

Disinfectants

H.1 Chemical Disinfectants

This appendix summarizes the pertinent characteristics and potential application of several types of commonly used chemical disinfectants. Because of the inherent hazards involved, use of these substances shall be reviewed in advance by the ES&H Team. For more information regarding disinfectants and required contact times, see Document 13.1. Table G-1 summarizes liquid, gaseous, and physical decontaminating agents for sanitation, disinfection, and sterilization.

H.1.1 Alcohol

A concentration of 70%–80% by weight of ethyl or isopropyl alcohol is commonly used as a general disinfectant. Alcohol denatures proteins and is a somewhat slow-acting germicide. Although a good general disinfectant, alcohol exhibits no activity against bacterial spores.

H.1.2 Chlorine

This halogen is a universal disinfectant that is active against all microorganisms and bacterial spores. Free, available chlorine is an active element. Chlorine combines with protein (e.g., organic matter) and rapidly decreases in concentration in its presence. It is a strong oxidizing agent that is corrosive to metals. If chlorine is used directly on a stainless surface, rinse thoroughly with water after decontamination to prevent tarnishing.

Chlorine solutions gradually lose their strength over time and therefore have a limited shelf life. Fresh solutions shall be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine disinfectant. For disinfecting purposes, a concentration of between 5,000 and 10,000 ppm of available chlorine is sufficient. Household or laundry bleach usually contains 5.25% available chlorine or 52,500 ppm. A 1:10 diluted solution contains 5,250 ppm of available chlorine. Decontamination with this concentration requires a 30-minute contact time. Do not autoclave chlorine solutions or materials treated with chlorine, because residual chlorine vaporizes, creating an inhalation hazard.

Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins.

Decontaminating agent	No effect on	Sanitize ^a	Disinfect ^a	Sterilize ^a
Liquids				
Alcohol, ethyl (70–95% v/v)	Bacterial spores ^b	—	TB ¹ at 95% for 0.25 min	—
			TB ² at 70% for 0.5 min	—
	Nonlipid viruses ^c	—	Lipoviruses for 1.0 min	—
	—	—	At 50% for 2.0 min	Medical instruments ^b at 70% for 15 min
Alcohol, isopropyl (60–90% v/v)	Bacterial spores ^d	At 70% for <2 min	At 70% for 2 min	At 70% for >5 min
Alcohol, Isopropyl +5% propylene oxide	—	—	—	Bacterial spores ^b for 1.0 min
Glutaraldehyde ^b	—	At <2% for 15 min	Bacillus anthrax at 2% for 15 min	At >2% for 15 min
	—	—	Cl. and Bacillus spores ^b at 2–3% for 180 min	—
Hydrogen peroxide	—	At <3% for 10 min	At 3–6% for 10 min	At 6–25% for 10
	—	—	10 ⁸ bacterial spores ^b at 10% for 60 min	—
Phenol (1–5%) ^c	Bacterial spores, ^c Nonlipid viruses	At 0.5% for <30 min	Broad spec. at 0.5–3% for 30 min C. burnetti, F. tularensis, P. mallei, Bacillus anthracis, and Rickettsia at 5% for 30 min	At >3% for 30 min

Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins. (cont'd.)

Decontaminating agent	No effect on	Sanitize ^a	Disinfect ^a	Sterilize ^a
Liquids (continued)				
Soap and water ^e	—	<i>C. burnetti</i> ^{f,g} for 10 min. The toxins will be diluted if washed for more than 10 min.	Synergistic with certain phenol derivatives	—
Sodium hypochlorite, (household bleach) undiluted	—	—	—	<i>Cl. botulinum</i> toxin, SEB, and Ricin ^g at 250 ppm (0.5%) for 15 min
	—	At <50 ppm (<0.1%) for 30 min	Most bacterial toxins at 50 ppm (0.1%) for 30 min TB at 50 ppm (0.1%) for for 30 sec at pH of 8.4 at 50–60°C General bacteria and viruses at 500 ppm (1.0%) for 30 min Bacillus anthracis ^b at 2500 ppm (5%) for 30 min	Lipoviruses at 500 ppm (1.0%) for 30 min
	—	—	—	SEB at 250 ppm (0.5%) for 72 min T2 mycotoxin at 2500 ppm (5%) for 72 min
Gaseous Agents				
Paraformaldehyde ^b	—	At <1% for 60 min	At 2% for 60 min	SEB ⁵ at >2% for 60 min
	—	—	<i>Cl. spores</i> and <i>Bacillus</i>	—

			spores at 8% for 180 min	
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Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins. (cont'd.)

Decontaminating agent	No effect on	Sanitize ^a	Disinfect ^a	Sterilize ^a
Gaseous Agents (continued)				
Ethylene oxide (12% EtO: 88% propellant)	—	—	—	At 130°F for 1200 mg/L for 120 min At 130°F at 650 mg/L for 240 min At 130°F at 470 mg/L for 320 min At 100°F at 470 mg/L for 480 min
Vapor-Phase Hydrogen Peroxide at 2 mg/L (20%)	Porous materials	—	—	B. globigii for 0.17 min Cl. sporogenes for 0.20 min B. Stearothermophilus for 0.30 min
Physical Agents				
Steam heat				
At 80°C	—	—	VEE, SEB, and Ricin for 30 min	—
At 121°C	(SEB) E. coli enterotoxin, Saxitoxin, and Tetrodotoxin	—	—	All bacteria, lipoviruses, protein-based toxins, and viruses for 15 min
At >500°C	—	—	T2 mycotoxin for 30 min	—
Gamma radiation (Cobalt 60)	—	—	—	S. faecium: <50 organisms/item at 3.2 Mrad; 50–500 organisms/item at 4.5 Mrad; and 500–5000 organisms/item at 5.0 Mrad

Table H-1. Summary of liquid, gaseous, and physical decontamination agents for biological agents and toxins. (cont'd.)

Decontaminating agent	No effect on	Sanitize ^a	Disinfect ^a	Sterilize ^a
Physical Agents (continued)				
Ultraviolet (UV) light (180–280 nm UV-C)	Cl. botulinum toxin, bacterial spores, Ebola virus, slow-growing viruses, lipoviruses, Micrococcus radiodurans, and non-protein-based toxins	At 0.5 mW/cm ² for 1440 min (24 h) (Ref. 1)	Biological safety cabinets, for example, at 35–40 m/W/cm ² for 30 min	T2 mycotoxin and protein-based toxins at >40 mW/cm ² for 30 min
X ray	Bacterial spores, Micrococcus radiodurans, and Micrococcus radiophilus	—	Salmonella typhimurium at 0.65 Mrad (6500 Gy)	Medical devices ^b at 2.5 Mrad (25,000 Gy)

^a Contact times may vary and are dependent on the concentration of agent used.

^b Seymour S. Block, *Disinfection, Sterilization, and Preservation*, 3rd edition, Lea & Febiger (1983), p. 1053.

^c American Industrial Hygiene Association, *Biosafety Reference Manual*, 2nd edition, Publication No. 204-RC-95 (1995), p. 175.

^d 59 FR 34496, *Guidelines for Research Involving Recombinant DNA Molecules*, July 5, 1994. Also see the following Internet address: <http://www.nih.gov/od/oba/rdna.htm/>

^e Soap and water will often dilute contaminants from equipment and skin.

^f Synergistic with sodium palmitate, sodium stearate, sodium ricinate, coconut soap, and castile soap.

^g Franz, David, *U.S. Army Defense Against Toxin Weapons*, 2nd edition, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD (August 1996).

^h *U.S. Army Field Manual 3-9, "Potential Military Chemical/Biological Agents and Compounds"* (December 12, 1990).

H.1.3 Iodophor

The most widely used groups of disinfectants in laboratories are iodophors (e.g., Wescodyne). Manufacturers recommend dilution ranges from 1 oz in 5 gal of water (i.e., 25 ppm) to 3 oz in 5 gal of water (i.e., 75 ppm or 0.0075%). The disinfectant characteristics of iodophor are similar to chlorine in that small amounts are rapidly taken up by extraneous protein. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if an appreciable amount of protein is present or if the pH is below 6 or above 7. For washing hands or for use as a sporicide, Wescodyne should be diluted to a concentration of 1:10 in 50% ethyl alcohol to yield 1,600 ppm of available iodine, for relatively rapid inactivation of all microorganisms.

H.1.4 Quaternary Ammonium Compounds

After years of testing and use, there is still considerable controversy about the efficiency of quaternary ammonium compounds (commonly referred to as “quats”) as disinfectants. These cationic detergents are strongly surface-active and are therefore good surface cleaners. Quats attach to protein and tend to clump microorganisms. Quats lose effectiveness in the presence of organic matter and are neutralized by anionic detergents such as soap. Quats are bacteriostatic, tuberculostatic, sporostatic, fungistatic, and algistatic at low concentrations. The compounds are biocidal against lipophilic viruses at medium concentrations but are virucidal only against hydrophilic viruses, even at high concentrations. Quats have the advantages of being odorless, nonstaining, noncorrosive to metals, stable, inexpensive, and relatively nontoxic. Caution should be used when handling concentrated quats; even a small droplet splashed into the eyes may cause damage to tissues. Be sure to wear safety glasses and proper PPE when handling these disinfectants. The concentration of the disinfectant used should be consistent with the manufacturer’s specification on the label.

H.1.5 Formaldehyde

Formaldehyde for use as a disinfectant is usually marketed as formalin solution (37% concentration of the gas in water) or as paraformaldehyde, a solid polymerized compound. Formaldehyde with an active ingredient of at least 5% is an effective liquid disinfectant but loses its disinfectant activity at 4°C or less. It is pungent with an irritating odor and is a suspected human carcinogen. Formaldehyde vapor generated from formaldehyde solution is an effective space disinfectant for sterilizing rooms or buildings. Formaldehyde gas can be generated by heating paraformaldehyde crystals. Formaldehyde vapors and gas are toxic and can elicit hypersensitivity and irritation. Dilution should be prepared in a chemical fume hood. Respiratory protection may be necessary. Formaldehyde gas is flammable and may be explosive under certain conditions.

H.1.6 Phenol

Phenol itself is not often used as a disinfectant. The odor is somewhat unpleasant, and a gummy residue remains on treated surfaces, especially when used in steam sterilization. Although phenols themselves may not be in widespread use, phenol homologs and phenolic compounds are the bases of a number of popular disinfectants. Phenolic compounds are effective disinfectants against some viruses, rickettsiae, fungi, and vegetative bacteria. The phenolics are not effective in ordinary use against bacterial spores.

Concentrated phenolics should be used carefully; even a small droplet splashed into the eyes may cause damage to tissues. Phenolics are readily absorbed by the skin, and splashes can cause local irritations, severe burns, and systemic poisoning, leading possibly to death. Therefore, safety glasses and other appropriate PPE should be worn. Contaminated skin should be washed aggressively with water.

H.1.7 Other Vapors and Gases

Ethylene oxide, peracetic acid, beta-propiolactone, methyl bromide, hydrogen peroxide, and ethylene amine are compounds that, when vaporized, are an effective disinfectant. When used in closed systems and under controlled conditions of temperature and humidity, excellent decontamination results can be obtained. Ethylene oxide, although a suspected carcinogen, is convenient to use, versatile, and noncorrosive. Peracetic acid is corrosive for metal and rubber. Beta-propiolactone, although a suspected human carcinogen, in the vapor state acts rapidly against bacteria, rickettsia, and viruses. It has a half-life of 3–5 hours when mixed with water, is easily neutralized with water and lends itself to removal by aeration.

H.2 Summary of General Precautions to Follow When Using Chemical Disinfectants

When handling concentrated stock solutions of certain disinfectants, be aware of the potential hazards, and exercise caution. For general information about chemical safety, see Document 14.1, "Chemicals," in the *ES&H Manual*. Concentrated quaternary and phenolic disinfectants are particularly harmful to the eyes. Even a small droplet splashed in the eyes may cause blindness. Absorption of phenolic compounds by the skin can lead to local irritation, severe burns, and systemic poisoning, leading possibly to death. Constant or prolonged exposure to phenol may cause headache, dizziness, difficulty in swallowing, diarrhea, vomiting, shock, convulsions, and death. Safety glasses and proper personal protective clothing should be worn to avoid corrosion and depigmentation of the skin. Good ventilation is required when working with phenol to minimize inhalation.

Vapors of formaldehyde can elicit hypersensitivity and irritation; they are also toxic, and working in a chemical fume hood is highly recommended. Respiratory protection may be necessary.

Pertinent characteristics and potential applications for several categories of chemical disinfectants most likely to be used in the biological laboratory are summarized in Table G-1.

The suggested practical concentrations and contact times may differ markedly from the manufacturer's recommendations. The efficacy of any of the disinfectants should be conclusively determined by individual investigators. In addition, wastes associated with the use of disinfectants shall be managed according to the requirements in Document 36.1.

Appendix I

Biological Safety Cabinets

Ventilation control of infectious agents or other biologically derived molecules is usually achieved with a BSC. The three primary classes of BSC are Class I, II, and III, which differ in design and containment capability.

The selection of an appropriate BSC for a given operation shall be approved by the area ES&H Team industrial hygienist according to the operation and an evaluation of the BSL classification. In addition, all BSCs shall be tested and evaluated by certified personnel after purchase and installation but before use; after being moved, relocated or serviced; and at least annually thereafter. Each class of BSC is described below (see Table I-1 for performance guidelines):

Class I Biological Safety Cabinets. Class I cabinets are similar to a conventional laboratory hood with an open-face, negative-pressure design. These cabinets are most suitable for BSL 1 and some BSL 2 and 3 containment.

Class II (Laminar Flow) Biological Safety Cabinets. Class II cabinets are used to protect the user from research materials and to protect the research materials from external contamination. Class II cabinets use HEPA filters as contamination controls. The downward flow of laminar air creates a protective barrier and prevents room air from contaminating the sterile work surfaces inside the cabinet. Room air is exhausted through the front grille. The internal cabinet air is exhausted through the front and rear grilles. Class II cabinets are further divided into four basic types (Type A, B1, B2, and B3), which differ in such respects as the:

- Proportion of air recirculated into the work area.
- Airflow velocities into the work opening and down toward the work surface.
- Manner of exhaust air discharge.
- Air plenum pressure relative to the room.

Class III Biological Safety Cabinets. Class III cabinets are hermetically sealed enclosures for the handling of extremely infectious materials.

Table I-1. Performance guidelines for biological safety cabinets.

Class	Description	Minimum face velocity (fpm) ^a	Pressure (inches w.g.) ^a	Hazard/risk level
Class I	Front panel not in place	75	NA	None to low
	Front panel without gloves	150	NA	None to low
	Front panel with gloves	NA	0.5 inch	None to low
Class II, Type A	Fixed height, usually 8" opening. 30% of air is HEPA filtered and exhausted into the room. 70% of air is HEPA filtered and recirculated into the work area. Viewscreen is hinged	A range of 100–110 fpm with an 8-inch opening	Positive pressure	Low to moderate risk with absence of volatile toxic chemicals and radionucleotides
Class II, Type B3	Telescoping legs or table top model equipped with a hard duct with thimble connection venting to the outside (30% exhaust, 70% recirculated through HEPA filters). Viewscreen is hinged.	A range of 100–110 fpm with an 8-inch opening.	Positive pressure	Low- to moderate-risk agents with minute quantities of toxic chemicals or trace amounts of radionucleotides
Class II, Type B1	Double HEPA filtered. 70% of air is HEPA filtered to the outside, 30% of the air is recirculated.	A range of 100–110 fpm with an 8-inch opening.	Negative pressure	Low- to moderate-risk agents with or without minute quantities of toxic chemicals or trace amounts of radionucleotides ^b
Class II, Type B2	Supply air is HEPA filtered. 100% of the air is HEPA filtered and exhausted to the outside.	A range of 100–110 fpm with an 8-inch opening.	Negative pressure	Low- to moderate-risk agents with or without toxic chemicals or radionucleotides ^b
Class III	Supply air is HEPA filtered. 100% of the air is doubled HEPA filtered.	NA	Negative pressure with 0.5-inch pressure drop	Very high risk. Access through double door sterilizer and decontaminant dunk bath.

^a Manufacturers provide specifications for BSCs with National Sanitation Foundation certification. The information in this table is not always applicable. Face velocity is measured in feet per minute (fpm).

^b The sliding sash is adjustable. Experiments should be performed with an 8-inch opening for proper face velocity.

General Operating Information

Understand how the BSC works, and plan your work. Protect yourself, your research, and your coworkers.

Keep your laboratory meticulously clean. Minimize storage of boxes and supplies, particularly near the BSC. Wash your hands thoroughly before and after working in the BSC. Wearing a clean laboratory coat and gloves while working in a BSC increases your safety and helps reduce contamination of research materials.

BSC effectiveness depends on proper directional airflow (i.e., inward and downward, through high efficiency filters) and can be reduced by anything that disrupts the air flow patterns, e.g., rapid movement of arms in and out of the BSC, people walking rapidly past the opening of the BSC, down-drafts from ventilation systems, and opened laboratory doors.

How to Use a Biological Safety Cabinet Effectively

- Turn off the BSC ultraviolet lamp if in operation and turn on the BSC. Wipe the work surface and the front window glass with the appropriate germicide (e.g., 70% ethanol or a 1:10 bleach solution). Wipe off each item you need for your procedure and place in the cabinet. Allow the cabinet to run for at least 5 minutes before beginning work.
- Do not place any objects over the front grille, and do not block the rear exhaust grille. Work should be performed at least 6 inches back from the front grille.
- Segregate contaminated items away from clean items. Minimize movement of contaminated items over clean ones. Remember to work from the clean side to the dirty side.
- Put on a laboratory coat, and thoroughly wash hands. Wear gloves, as appropriate.
- Follow good microbiological techniques, such as holding open tubes and bottles as horizontal as possible. Use convenient mechanical pipetting aids. Do not mouth pipette. Place horizontal pipette discard pans containing appropriate disinfectant or water inside the BSC. Do not place vertical pipette discard canisters on the floor outside the BSC.
- The heat from the open flame creates turbulence in airflow and compromises sterility; heat buildup may damage the filters. If flaming is required, use a Bunsen burner with a pilot light or an electric loop incinerator.

- If you need to remove items from the BSC or add new items, move your arms slowly in and out of the cabinet to minimize airflow disruption.
- If you need to use a piece of equipment that creates turbulence in the BSC (e.g., centrifuge, blender, and sonicator), place equipment in the back third of the cabinet, and stop work while equipment is operating.
- Protect the building vacuum system from contamination by placing a cartridge filter between the vacuum trap and the source. See Appendix H for more information.
- Clean up any spills in the BSC immediately. See Appendix F for more information and guidance.
- Remove all materials and wipe all interior surfaces with 70% alcohol when you finish work. Let the cabinet run for 10 minutes, then turn it off. Examine the tray under the work surface. Disinfect and clean as necessary.
- Segregate waste by category and then properly dispose.
- Remove laboratory coat and wash hands thoroughly before leaving the laboratory.

Reference: NIH Laboratory Safety Monograph, "A Supplement to the NIH Guidelines for Recombinant DNA Research," July 1978.

Appendix J

Vacuum Line Filters

The aspiration of tissue culture media from monolayer cultures or of supernatants from centrifuge samples into primary collection flasks is a common laboratory procedure. Protection should be provided against aerosols of hazardous chemical or biological materials and against the overflow of fluid into the vacuum system. This protection is provided by the use of an air filter in the line immediately leading into the house vacuum line and an overflow flask for liquids between the collection flask and the air filter.

To assemble this protective apparatus (see Figure J-1), use flexible tubing of appropriate inside diameter for the flask and filter fittings and of sufficient wall thickness for the applied vacuum. Filter flasks of capacities from 250 to 4000 ml may be used for the overflow flask, depending on the available space and the amount of fluid that could be accidentally aspirated out of the collection flask. The overflow flasks should contain a disinfectant solution appropriate for the biohazardous material under study. It is essential that an antifoam agent (e.g., Dow Corning Antifoam A) be added to the overflow flask, because bubbling of air through the disinfectant will cause considerable foam that, if allowed to reach the filter, will shut off the vacuum.

If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. Before the old filter is discarded, both the old filter and flask should be autoclaved, after which a new filter can be installed and the assembly replaced. A cartridge-type HEPA filter removes airborne particles 300 nm (0.30 micron) in size or smaller and therefore provides an effective barrier to the passage of aerosols into the house vacuum system.

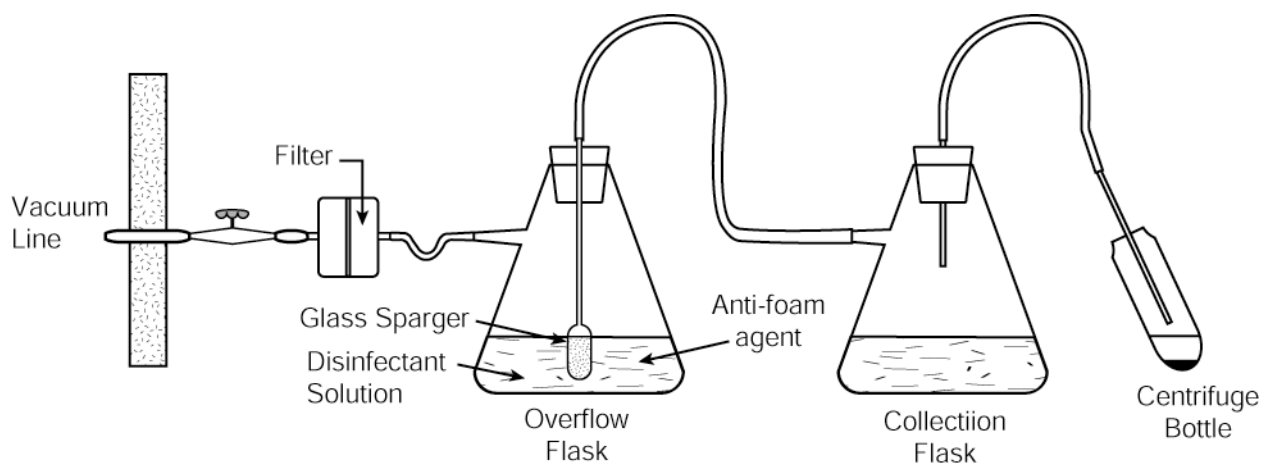


Figure J-1. Protective apparatus for vacuum system.